Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 11, pp 2029–2040, 1997

PII: S0968-0896(97)00141-7
0968-0896/97 $17.00 + 0.00

TRH Mimetics: Differentiation of Antiamnesic Potency from Antidepressant Effect†

Anatoly A. Mazurov,*,‡,[a] Sergei A. Andronati,[a] Tamara I. Korotenko,[a] Nikolai I. Sokolenko,[a] Alexei I. Dyadenko,[a] Yury E. Shapiro,[a] Vitalii Ya. Gorbatyuk,[a] and Tatyana A. Voronina[b]

[a]*Physico-Chemical Institute, National Academy of Sciences, Odessa, Ukraine*

[b]*Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia*

Abstract—For the purpose of rational modification of the TRH molecule, we were pursuing an approach that consists of two steps: (1) 'obligatory' replacement of histidine with glutamine in TRH and (2) the application of conformational constraints for putative bioactive conformation I stabilized by an intramolecular hydrogen bond between C-terminal carboxamide proton and α-carbonyl of histidyl (glutaminyl), and conformation II formed by an intramolecular hydrogen bond between α-carbonyl of pyroglutamyl and prolinamide proton. Significant antiamnesic potency was discovered in the passive avoidance test (ECS and Scopolamine induced amnesia) for conformation II mimic (8*S*,10a*S*)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (**2**) at doses of 0.1 and 1.0 mg/kg. EEG analysis indicates a mild activating effect of compound **2** on EEG, which is similar to that of piracetam and differs from hard amphetamine activation. Conformation I mimic 3-(2-carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo[1,2-*a*]pyrazin-1,4-dione (**1**) exhibited an antidepressant effect at a dose of 1 mg/kg. The transition from two putative quasi-cyclic bioactive conformations of TRH and its obligatory similar analogue [Gln^2]-TRH to their cyclic mimics led to differentiation of antiamnesic and antidepressant activity of TRH. © 1997 Elsevier Science Ltd.

Introduction

Thyrotropin-releasing hormone (TRH, Glp-His-Pro-NH_2)[1] was the first hypothalamic hypophysiotropic hormone to be isolated and characterized.[2,3] This short peptide displays dual functions, acting as both a hormone and a neuropeptide. Although TRH was originally classified as a hormone releasing prolactin and thyrotropin from the pituitary, it has characteristics of a CNS-activating substance functioning either as a neurotransmitter or as a facilitative neuromodulator.[4] This characterization is based primarily on the peptide's analeptic properties and ability to reverse the sedation and hypothermia induced by pentobarbital, ethanol, and diazepam.[5] TRH potentiates many of the neurotransmitter systems implicated in memory storage and retrieval, independently of its hormonal activity, and is effective in ameliorating some forms of memory disruption.[6] It has also been reported that thyroliberin improves physiological conditions and survival through cardiovascular, gastrointestinal and respiratory effects.[7,8] TRH-related alterations are associated with the pathophysiologies of various disease states including Alzheimer's disease,[9,11] depression,[12,13] schizophrenia,[14] epilepsy,[15,16] and endocrine or metabolic disorders.[17]

The TRH receptor (TRH-R) belongs to the family of seven-transmembrane-domain G protein-coupled receptor proteins. The existence of TRH in different regions throughout the brain, coupled with receptor binding sites in these regions, suggests the significance of thyroliberin for CNS function. While endocrine activity of TRH analogues correlates with TRH-R binding, discrepancy between their affinity and inhibition of haloperidol catalepsy,[18,19] stimulation of phosphoinositide turnover[20,21] and activity in behavioral models of cognition[22,23] support previous suggestion about existence of low-affininty TRH-receptors which bind CNS active analogues.[24]

For the purpose of rational modification of the TRH molecule, we were pursuing an approach based on our assumption about the existence of obligatory similar amino acids. Each natural amino acid is encoded by the triplets of nucleotides codons. As obligatory similar amino acids, we considered pairs of amino acids encoded by the same obligatory nucleotides (Table 1). According to Crick's wobble hypothesis, the first two codon bases (obligatory nucleotides) make the most significant contribution into the specific encoding in comparison with the third base (facultative nucleotide).[25] From 20 proteinogenic amino acids, seven pairs of obligatory similar amino acids might be elicited. Since obligatory similar amino acids are encoded by the same obligatory nucleotides we hypothesized that they

*To whom correspondence should be addressed.
†A preliminary communication has been reported: Mazurov A. A.; Andronati S. A.; Korotenko T. I.; Sokolenko N. I.; Dyadenko A. I.; Shapiro Y. E.; Gorbatyuk V. Ya.; Voronina T. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2595.
‡Present address: Astra Hässle AB, Medicinal Chemistry Department, Preclinical R & D, Mölndal, S 431 83, Sweden

can replace each other with the preservation of some kinds of biological effects in certain examples. Indeed, there are some natural occurring peptides that have similar biological potency and those peptides contain obligatory similar amino acids (e.g., magainins,[26] calcitonins,[27] growth hormone-releasing factor,[28] and angiotensin analogues,[29] Fig. 1).

Results and Discussion

Previously, the application this hypothesis of obligatory similar amino acids has been demonstrated for the neurohormone MSH release inhibiting factor (Pro-Leu-Gly-NH_2, MIF, melanostatin).[30,31] According to the above-mentioned hypothesis, the second amino acid of melanostatin leucine was exchanged with phenylalanine. Both peptides, MIF and [Phe^2]-MIF showed a similar profile of locomotor activity after intracerebroventricular administration.[32] The level of major neurotropic effect of MIF, antidepressant activity, was approximately retained after obligatory replacement of leucine.[30] Nevertheless, previous endeavors to change the second amino acid of MIF caused a sharp alteration of the psychotropic effect up to its disappearance.[33] To identify the influence of the obligatory replacement on peptide conformation, we determined the spatial structure of melanostatin and its analogues using 2-D NMR conformational analysis.[31] It was revealed melanostatin and its [Phe^2]-analogue exist in DMSO solution in conformation of β_{II}-fold, which is stabilized by an intramolecular hydrogen bond. Analysis of the spatial structure of MIF analogues allowed us to conclude that the obligatory replacement of leucine with phenylalanine weakly affects the ϕ dihedral angle values and does not change the β_{II}-turn. However, incorporation of either electron-withdrawal or electron-release substituents in the aromatic ring of phenylalanine alters the ϕ angles for the second amino acid, which lead to the slackening of the intramolecular hydrogen bond and enhances the plausibility of distortion of β_{II}-conformation.

GIGKFLHSAGKFGKAFVGEIMKS *I*
GIGKFLHSAKKFGKAFVGEIMNS *II*

Magainins

CASLSTCVLGKLSQELHKLQTYPRTDVGAGTP-NH_2 *chicken*
CSNLSTCVLGKLSQELHKLQTYPRTDVGAGTP-NH_2 *eel*
CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP-NH_2 *human*
CGNLSTCMLGTYTQDLNKFHTFPQTASGVGAP-NH_2 *rat*
CSNLSTCVLGKLSQDLHKLQTYPRTNTGSGTP-NH_2 *salmon*

Calcitonins

YADAIFTNSYRKVLGQLSARKLLQDIMSRNNGESNQERGA *human*
HADAIFTSSYRRILGQLYARKLLHEIMNRNNGERNQEQSRFN *rat*

Growth Hormone Releasing Factor

DRVYIHPF *Angiotensin II*
ERVYIHPF *Glu^1-Angiotensin II*

Figure 1. Examples of biologically active peptides containing 'obligatory' similar amino acids.

Analogueous obligatory replacement of second amino acid histidine by glutamine was undertaken for thyroliberin. Pyroglutamyl-glutaminyl-prolinamide (**10**)[34] retains the antiamnesic activity of TRH without exhibition of any antidepressant effect.

2-D NMR conformational analysis

Both TRH and [Gln^2]-TRH have great conformational similarities (Fig. 2). Conformations of TRH and Glp-Gln-Pro-NH_2 in $(CD_3)_2SO$ solution were determined using 2-D ^{1}H NMR spectroscopy (δ-J correlated, COSY, and NOESY) in accordance with the approach applied to the MIF analogues.[31] Dihedral angles (ϕ_i, ψ_i of the backbone chain and χ_i^1, χ_i^2 of the side chain) that characterize the spatial structure of a peptide, are given in Table 2. *Trans* configuration of the histidyl-prolyl peptide bond in TRH was deduced from cross peaks caused by dipole–dipole interaction between protons $C^{\alpha}H$, $C^{\beta}H_2$ of histidyl residue, and protons $C^{\delta}H_2$ of prolyl residue. In addition, cross peaks between protons $C^{\alpha}H$, $C^{\beta}H_2$ of histidyl residue, and protons $C^{\alpha}H$ of prolyl residue correspond to *cis* isomerism of this molecule. *Cis–trans* isomerism of proline in TRH was first detected by Deslauriers and co-workers using ^{13}C NMR spectroscopy.[35] The opportunities for typical dipole-dipole interaction between the imidazole ring of histidine and the carboxamide were discussed as well.[36] We observed cross peaks between the carboxamide protons and protons $C^{\alpha}H$, $C^{\beta}H_2$ of histidyl residue. This interaction is possible for dihedral angles $\psi_2 = 0°$, $\chi_2^1 = -125°$, $\omega_3 = 180°$, and $\psi_3 = 0°$. Formation of the intramolecular hydrogen bond between the carbonyl of the histidyl–prolyl peptide bond and the carboxamide group is likely for angles $\omega_3 = 180$

Table 1. 'Obligatory' similarity of amino acids

Asp	GAU	GAC	
Glu	GAA	GAG	
Phe	UUU	UUC	
Leu	UUA	UUG	
	CUA	CUG	
	CUU	CUC	
His	CAU	CAC	
Gln	CAA	CAG	
Asn	AAU	AAC	
Lys	AAA	AAG	
Cys	UGU	UGC	
Trp	UGG		
Ile	AUU	AUC	AUA
Met	AUG		
Ser	AGU	AGC	
Arg	AGA	AGG	
	CGA	CGG	
	CGU	CGC	

Table 2. Dihedral angles (deg) for amino acid residues in Glp-His-Pro-NH_2 and Glp-Gln-Pro-NH_2 according to NOESY data

Peptide	Amino acid residue	ϕ	ψ	χ^1	χ^2
TRH	Glp	120	−30	—	—
			180		
	His	−89	0	±125	0
		−152	170		180
	Pro	—	0	—	—
10	Glp	120	60	—	—
			160		
	Gln	−88	120	±126	±60
		152			180
	Pro	—	0	—	—

ϕ, ψ, χ^1, χ^2 — dihedral angles around N-C^α, C^α-CO, C^α-C^β, and C^β-C^γ bonds for amino acid residues.

and $\psi_3 = 0$. Noteworthy is the prevalence (94%) of *trans*-configuration of the histidyl–prolyl peptide bond, which might be explained by the hydrogen binding.[37]

Two forms differing in the *cis–trans* isomerism of the glutaminyl-prolyl peptide bond were detected for the TRH analogue pyroglutamyl-glutaminyl-prolinamide (Glp-Gln-Pro-NH_2). Based on the integral intensity of double peaks for NH, $C^\alpha H$ of glutaminyl, $C^\delta H$ of prolyl and C-terminal carboxamide protons, the ratio of *trans*:*cis* isomers was estimated as 4:1. The proximity of the *syn* carboxamide proton and α-carbonyl of glutaminyl occurs for *trans* glutaminyl-prolyl peptide bond and $\psi_3 = 0°$. Formation of the intramolecular hydrogen bond between the α-carbonyl of pyroglutamyl and the C-terminal carboxamide proton is feasible for *cis*-isomer with dihedral angles $\phi_2 = -88°$, $\psi_2 = 120°$, $\psi_3 = 0°$. Observed *d*-contacts between *syn*/*cis* carboxamide proton and pyroglutamyl $C^\alpha H$ in NOESY spectra support this assertion. The energy of hydrogen bond formation was calculated using the Arrhenius equation and was based on temperature dependence of spin-lattice relaxation time, T_1, for flexible protons. For the *cis*-isomer, the hydrogen bond energy is 24.3 KJ/mol and for the *trans*-isomer it is 20.1 KJ/mol. Experimental data are consistent with results of theoretical conformational analysis conducted using Allinger's MM2 force field.[38]

Earlier,[39] it was supposed that TRH takes a conformation with an intramolecular hydrogen bond between the carboxamide hydrogen and α-carbonyl of pyroglutamyl after recognition by the receptor. Twenty percent of [Gln^2]-TRH molecules exist in a quasicyclic conformation in solution. Transition from the bioactive conformation of the peptide to its mimic might maximize the potential advantages, which are realized with the application of conformational constraints.

In order to stabilize the putative bioactive conformations of TRH and its obligatory similar analogue Glp-Gln-Pro-NH_2 with intramolecular hydrogen bonds between C-terminal carboxamide proton and α-carbonyl of histidyl (glutaminyl) (conformation I) or α-carbonyl of pyroglutamyl (conformation II), we synthesized the two heterocycles: (3*S*,8a*S*)-3-(2-carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo-[1,2-*a*]pyrazin-1,4-dione (**1**) and (8*S*,10a*S*)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]-diazocin-5,10-dione (**2**). Since a histidine residue in TRH was replaced with an obligatory similar glutamine, a side chain moiety of glutamine was incorporated into the designed compounds. The position of the carbamoyl group in the pyrrolodiazocine ring was determined using the distance between nitrogen atoms of glutamine side chain and proline in minimum-energy conformations of pyroglutamyl-glutaminyl-prolinamide (**10**). The interatomic distance, N^α_{Pro}–N^δ_{Gln} is 5–6Å for peptide **10** and N^4–$N_{carbamoyl}$ is 5.5Å for its mimic **2**.

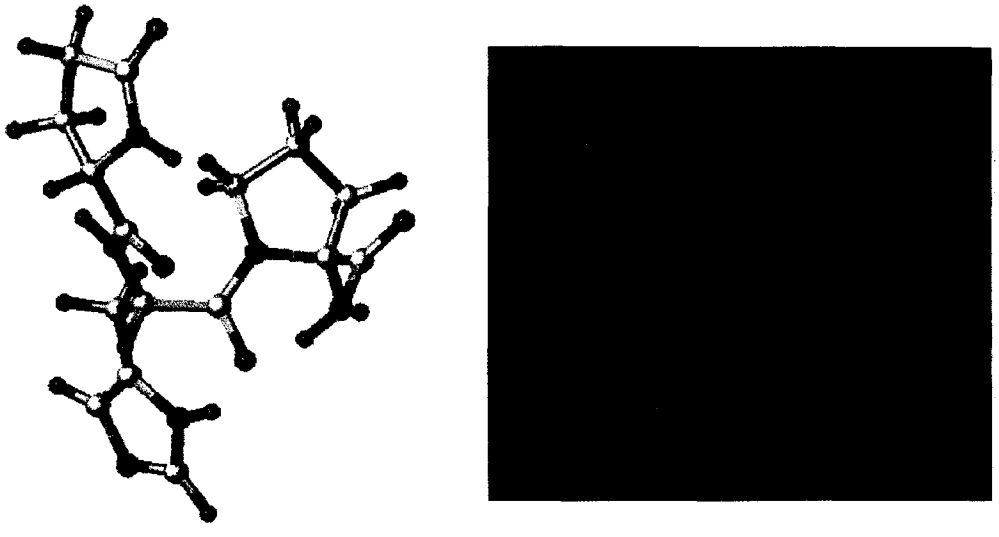


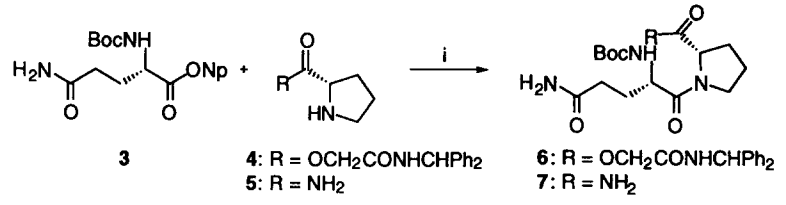
Figure 2. Conformations of Glp-His-Pro-NH_2 (TRH) (left) and Glp-Gln-Pro-NH_2 (**10**) (right) in DMSO-d_6 determined by two-dimensional NMR conformational analysis. The peptides contain obligatory similar amino acids histidine and glutamine.

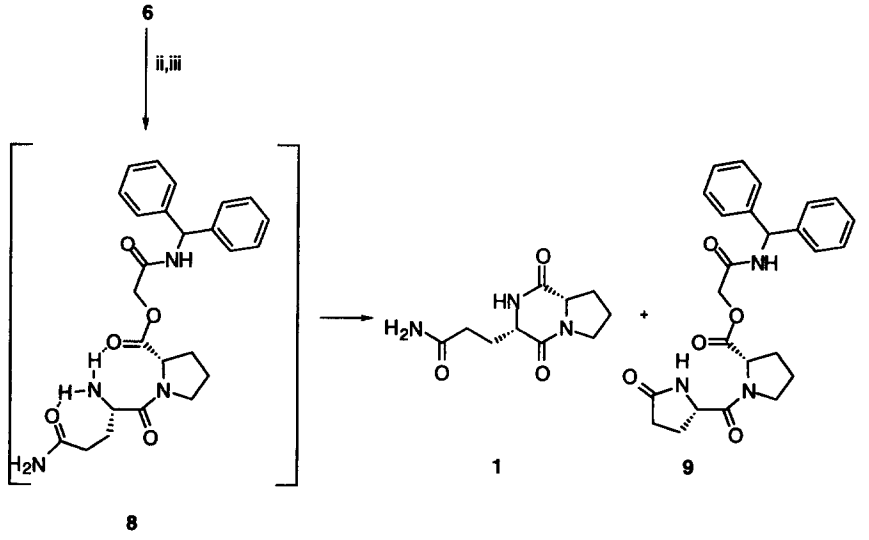
Chemistry

To ensure sufficient ring closure at mild conditions, glutaminyl-proline *N*-benzhydrylglycolamide ester (**8**) containing more electrophilic carbonyl in comparison with methyl or ethyl esters was carried out by condensation of *N*-protected glutamine active ester **3** with proline *N*-(diphenylmethyl)carbamoylmethyl ester (**4**)[40] followed by acidolytic cleavage of the *tert*-butyloxycarbonyl group (Scheme 1). After deprotonation of the amino moiety, the unstable peptide base **8** was readily cyclized at room temperature. Besides diketopiperazine, the unexpected side product pyroglutamyl-proline *N*-benzhydrylglycolamide ester (**9**), was also isolated.

3-(2-Carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo[1,2-*a*]pyrazin-1,4-dione (**1**) also appeared as a major product in the conventional step-by-step method of peptide synthesis of pyroglutamyl-glutaminyl-prolinamide (**10**),[41] where prolinamide (**5**) was coupled with *p*-nitrophenyl ester **3**. After deprotection of dipeptide **7**, glutaminyl-prolinamide was acylated by pyroglutamic acid activel ester to afford only 16% of the desired peptide **10** (Scheme 2). Pyroglytamyl-glutaminyl-prolinamide was successfully obtained in a good yield after a change in the synthesis strategy. N^{α}-Protected glutamine *N*-benzhydryl-glycolamide ester (**12**) was obtained from *N*-*tert*-butyloxycarbonyl glutamine (**11**). After acidolytic cleavage of N^{α}-*tert*-butyloxycarbonyl group glutamine ester reacted with pyroglutamic acid pentafluorophenyl ester to afford dipeptide **13**. After saponification of *N*-(diphenylmethyl)carbamoylmethyl ester **13**, pyroglutamyl-glutamine was coupled with prolinamide by carbodiimide method to give pyroglutamyl-glutaminyl-prolinamide (**10**). Little, if any, racemization occurred during this route. Although the risk of racemization of dipeptides is much greater than that of monomers, no evidence of this, or any other significant impurity, was observed in both HPLC and NMR.

The unusual easy ring closure of glutaminyl-prolinamide and the formation of a pyrrolidone ring by intramolecular attack of the glutamine γ-carbonyl by amino nitrogen in the presence of more electrophilic ester carbonyl during cyclization of glutaminyl-proline *N*-benzhydrylglycolamide ester (**8**), prompted an assumption about acquired high reactivity of the α-amino group of glutamyl residue. Apparently, the amino moiety may be involved simultaneously in two hydrogen bonds with the γ-carbonyl of glutamyl residue and the α-carbonyl of proline residue forming a quasicluster structure. Due to activation through intramolecular quasicluster hydrogen bonding, the electrophilicity of both carboxamide carbon atoms and the nucleophilicity of the amino nitrogen atom are greatly enhanced.



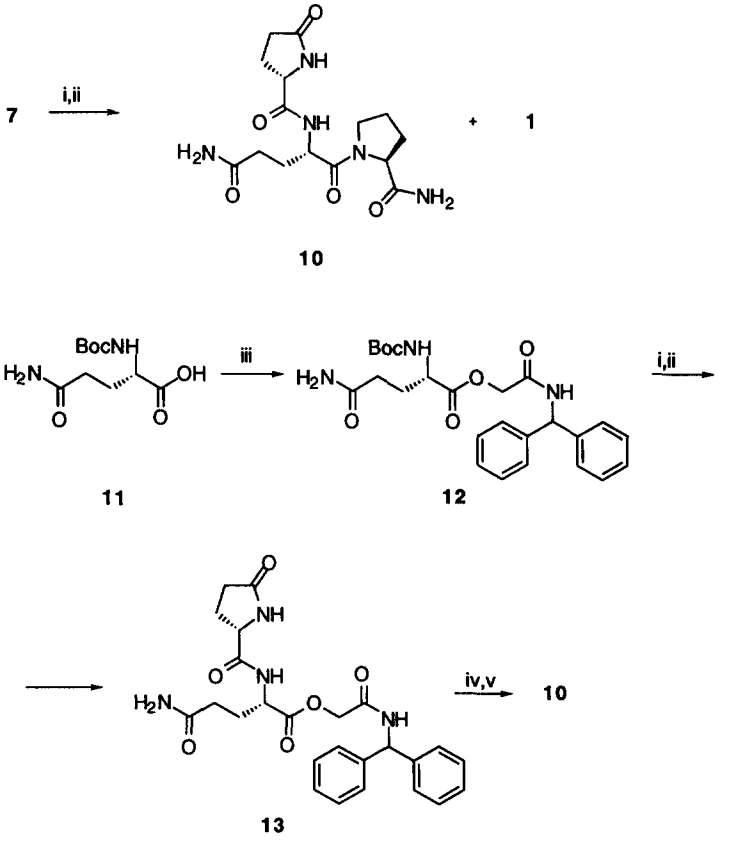


Scheme 1. (i) BtOH, DMF; (ii) HCl/dioxane; (iii) *i*-Pr_2NEt, DMF.

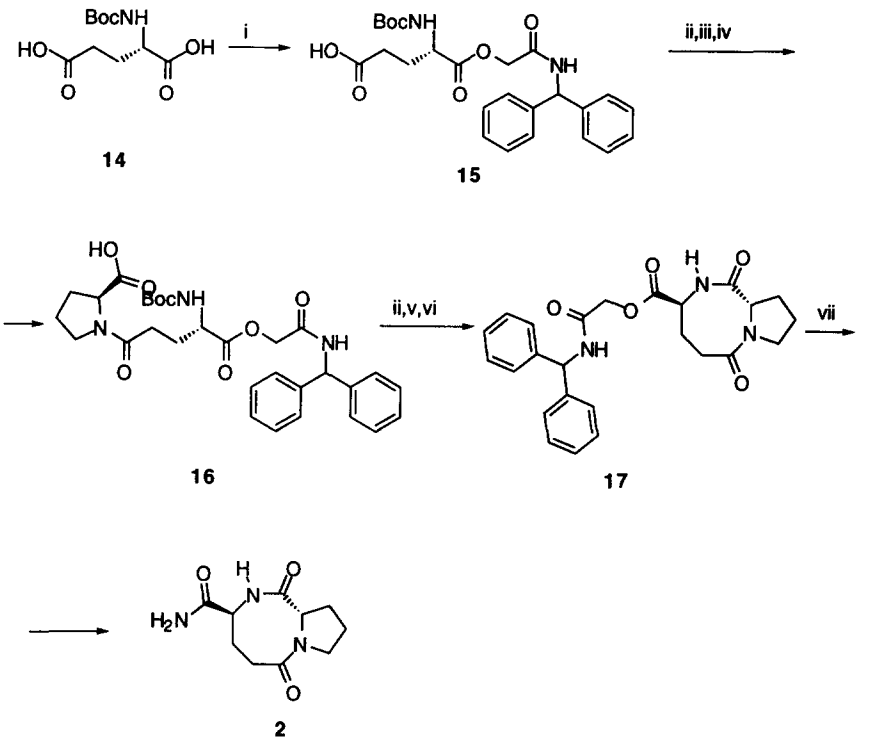
The perhydropyrrolo[1,2-*a*][1,4]-diazocine ring system, which imitated a quasicyclic conformation II stabilized by intramolecular hydrogen bond between proline carboxamide proton and α-carbonyl of pyroglutamyl residue, was obtained starting from *N*-*tert*-butyloxycarbonyl glutamic acid (**14**) (Scheme 3). The α-carboxyl group was selectively blocked by conversion into *N*-benzhydrylglycolamide ester **15** using the substantial difference in acidity of α- and γ-carboxyl moieties. Application of crown ethers, forming soluble inorganic solvent complexes with alkaline metal salts of carboxylic acids, allows esterification in a homogeneous phase in mild conditions.[42] A reaction of α-amino and α-carboxyl protected glutamic acid **15** with proline was performed by the modified method of salt condensation.[43] The carboxyl function of proline was blocked by its transformation into a complex of proline sodium salt with 15-crown-5. A solution of the latter in *N*,*N*-dimethylformamide was treated by *N*-*tert*-butyloxycarbonyl glutamic acid α-benzhydrylglycolamide ester -*N*-hydroxysuccinimide ester to afford dipeptide **16** after neutralization of its sodium salt with acetic acid. In order to form an eight-member ring, the active ester method was applied. The γ-carboxyl group of glutamyl-(α-benzhydrylglycolamide ester)-proline (**16**) was activated by formation of a *N*-hydroxysuccinimide ester. Deprotection of the α-amino moiety led to ring closure and the formation of pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione **17**. A hydrophobic diphenylmethyl fragment facilitates the isolation and purification of the heterocycle. Meanwhile, the benzhydrylglycolamide ester moiety of **17** was readily converted into an amide by ammonolysis in dioxane to afford the target compound **2**.

Pharmacology

New compounds were tested for the antidepressive and antiamnesic effects of TRH on the CNS of rats after intraperitoneal administration. Although the therapeutic efficacy of TRH for depression treatment found in earlier studies[44,45] had not been confirmed later[46–48], it was revealed that tricyclic antidepressants exhibited their action via allosteric effects on the TRH receptors of the CNS[49,50] and TRH had a CNS-action similar to that of amitriptyline in an experimental model of behavioral depression.[51] The antidepressive potency of synthesized compounds was examined in an experimental model of behavioral depression described by Porsolt et al.[52] The search for new antiamnestic drugs generally relies on behavioral tests, since the mechanism of action of cognition enhancers is still under investigation.[53,54] Cognition enhancing activity was assessed by the passive avoidance test, widely used for screening purposes, with the reversal of the experimentally induced by either electric convulsive shock (ECS) or scopolamine amnesia.[55,56]



Scheme 2. (i) 4 N HCl/dioxane; (ii) Glp-OPfp, *i*-PrNEt, DMF; (iii) $BrCH_2CONHCHPh_2$, CH_3Ona, DMF; (iv) aq Na_2CO_3; (v) BtOH, DCC, H-Pro-NH_2.



Scheme 3. (i) CH_3ONa, 15-crown-5, $BrCH_2CONHCHPh_2$, EtOAc; (ii) DCC, SuOH, THF; (iii) H-Pro-ONa, 15-crown-5, DMF; (iv) AcOH; (v) 4 N HCl/dioxane; (vi) *i*-Pr_2NEt, MeCN; (vii) NH_4OH.

The mimetic of conformation I for pyroglutamyl-glutaminyl-prolinamide diketopiperazine **1** showed high antidepressive activity at doses of 1.0 and 0.1 mg/kg (Table 3). Significant antiamnesic potency was discovered in the passive avoidance test for the conformation II mimic **2**. (8*S*,10a*S*)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (**2**) completely reversed ECS induced amnesia (Table 4). The different types of activity of the structural isomers are mimetics of two conformations of the biologically active peptide, which allows us to draw conclusions about the possibility of the differentiation of antidepressive and antiamnesic effects of TRH. Since the amnesic effect of scopolamine has been attributed to a central cholinergic action[57,58] and cholinergic impairment is associated with the aging of the brain and senile dementias, we evaluated the ability of diazocine to protect rats from scopolamine-induced amnesia in the passive avoidance test. This compound is able to reverse scopolamine-induced amnesia at a level of 83% at a dose of 1 mg/kg (Table 4), whereas oxiracetam[59] shows only a 12% reversal of this deficit at the same dose. In the acute hypobaric hypoxia model (Table 5), the compound showed antihypoxic activity in the dose of 0.1 mg/kg. Also, it was observed that the tendency of animals to survive increased for doses of 1 and 5 mg/kg. The new compounds **1** and **2** had no sedative and myorelaxant action, motor impairment or psychomotor activation on the CNS in neuropharmacological tests in mice.

Quantitative electroencephalography (EEG) makes it possible to discriminate the effects of different drugs, and may therefore be a useful means of analyzing changes in the EEG of animals induced by substances acting on the CNS.[60] Visual analysis of the electroencephalogram does not reveal any action of compound **2** on the cortex. Statistically significant effects can only be determined using computer assisted EEG analysis (Tables 6 and 7). The degree of EEG modification by this compound is comparable to that of piracetam and is surpassed significantly by amphetamine response. The pattern of EEG-parameter changes indicates a mild activating effect by compound **2** on EEG, which is similar to that of piracetam and differs from hard amphetamine activation.

Recently, Cosentino et al.[61] identified a common spatial disposition of the polar functional groups present in a series of piracetam-type nootropics and suggested two

Table 3. Antidepressant effect

Compound	Immobility time (%[a])	
	1 mg/kg	0.1 mg/kg
TRH	82±7	80±10
1	57±9**	75±12
2	81±17	112±13
10	128±7**	114±8
Pyrazidol	88±7*[b]	

[a]Immobility time of control group was counted for 100%.
[b]Dose 25 mg/kg of pyrazidol.
*$p<0.05$.
**$p<0.01$.

Table 4. Amnesia-reversal activity

Compound	Amnesic agent	Amnesia reversal (%)		
		5 mg/kg	1 mg/kg	0.1 mg/kg
TRH	ECS	—	76*	23
Aniracetam	ECS	—	29*	—
Aniracetam	Scopolamine	—	18	—
1	ECS	—	17	12
2	ECS	—	100*	100
2	Scopolamine	48	83**	52
10	ECS	—	23	10

*$p<0.05$.
**$p<0.01$.

possible pharmacophoric models. Using conformational analysis and chemometric methods, three pairs of the interatomic distances (D_{O-O} = 3.69, 3.61, D_{C-C} = 3.17, 3.23, and D_{N-X} = 3.02, 3.77) within the polar groups (an N–C=O amide group and a X–C=O group with X=O, N) present in the 2-pyrrolidone-containing nootropics were determined. Later, the proposed molecular determinants of amnesia-reverting activity were corroborated and supplemented by X-ray diffraction, NMR spectroscopy, ab initio and high-temperature-quenched molecular dynamic calculations data.[62] Both pyrrolo-[1,2-*a*]1,4-diazocine **2** and prolylglutaminyl diketopiperazine (**1**) contain three amide groups as possible pharmacophores. Molecular modeling was performed in order to verify whether the discovered antiamnesic compound might fit the pharmacophore models previously defined. Table 8 reports distances D_{O-O}, D_{C-C}, and D_{N-N} calculated for minimum-energy conformers of these molecules. The distances between atoms of two amide moieties $D_{O^1-O^2}$ = 3.73 Å, $D_{C^1-C^2}$ = 3.05 Å, and $D_{N^1-N^2}$ = 3.44, for the antiamnesic compound coincide with pharmacophores proposed for 2-pyrrolidone-containing nootropics. Whereas the geometry of a low-energy diketopiperazine conformation cannot adopt distance requirements for amide groups in nootropic pharmacophoric model. Although compound **2** has no pyrrolidone fragment, which is an important pharmacophore feature for classical nootropic drugs like piracetam and its analogues, the fair fitting of spatial disposition of amide groups in pyrrolidino[1,2-*a*]1,4-diazocine to piracetam-type nootropics would expand the significance of the proposed pharmacophore model in the design of new cognition enhancers.

Conclusion

Taken together, the results with 'obligatory' replacement of amino acids in small biologically active peptides MIF and TRH forming quasicyclic conformations, which are stabilized by a hydrogen bond, demonstrate the application of the proposed approach for purposeful design of new active compounds. The transition from two putative bioactive conformations of TRH and its obligatory similar analogue [Gln^2]-TRH to their mimics 3-(2-carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo[1,2-*a*]pyrazin-1,4-dione (**1**) and (8*S*,10a*S*)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo-[1,2-*a*][1,4]diazocin-5,10-dione (**2**) led to the differentiation of antiamnesic and antidepressant effects of TRH, and originated new classes of cognitive enhancers and antidepressants.

Table 5. Antihypoxic effect of **2**

	Saline	0.1 mg/kg	1 mg/kg	5 mg/kg
Number of rats	16	16	16	8
Number of survived rats	3	9	6	2
% of survived rats	18.8±10.1	56.3±12.8*	37.5±12.5	25.0±16.4

*$p<0.05$.

Table 6. Mahalanobis' distances for EEG after drug administration

Period after drug administration (min)	Amphetamine (1 mg/kg)	Piracetam (250 mg/kg)	2 (1 mg/kg)	2 (5 mg/kg)
0–30	4.63**	0.91*	0.50	1.73**
30–60	6.96**	0.70*	0.54*	1.29**
60–90	4.03**	0.59*	0.57*	0.92*

*$p<0.05$
**$p<0.01$

Table 7. Alterations of amplitude-interval EEG parameters in 30–60 min after administration of **2**[a]

EEG parameters	Norepinephrine (1 mg/kg)	Piracetam (250 mg/kg)	2 (1 mg/kg)	2 (5 mg/kg)
F	1.91±0.76	2.40±0.70**	0.52±0.66	2.74±0.77**
A	−25.3±2.3**	−10.2±2.4**	−4.5±2.5	−12.3±2.5**
F_β	5.33±0.48**	1.53±0.41*	0.57±0.41	3.07±0.45**
I_θ	14.7±1.4**	2.3±1.1*	1.1±1.3	2.5±1.1*
I_α	−14.8±1.8**	−5.5±1.7**	−5.3±2.0**	−2.9±1.9
I_β	−0.9±1.7	3.7±1.5*	1.4±1.3	2.3±1.6*

[a]Differences of mean EEG values for experimental and control groups of animals are indicated. Original parameters for each animal were expressed in percent towards base level which was taken for 100%.
Abbreviations: F—total average frequency; A—total average amplitude; I_α, I_β, I_θ—time indexes for α, β, θ bends of EEG; F_β—average frequency of β bend.
*$p<0.05$.
**$p<0.01$.

Experimental

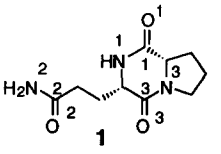
Chemistry

Optical rotation was measured with a Perkin–Elmer 241 MC polarimeter. Proton magnetic resonance spectra were obtained on a Bruker 250-MHz spectrometer in DMSO-d_6; chemical shifts were expressed in δ values downfield from tetramethylsilane; the coupling constants are expressed in hertz. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; m, multiplet. Melting points were determined on a electrothermal apparatus and are uncorrected. Elemental analyses were agreed with theoretical values within 0.40%. Homogeneity of all compounds was checked by TLC and HPLC. HPLC was performed on a Du Pont model 8800 instrument using a Zorbax C8 analytical column with detection at 210 nm. For TLC on precoated plates, Silufol (Kavalier) and Kieselgel 60F-254 (Merck) were used with the following solvent systems: (a) benzene:acetone:acetic acid, 100:50:1; (b) chloroform:ethyl acetate:methanol:acetic acid, 9:3:2:1; (c) butanol:acetic acid:water, 4:1:1; and (d) chloroform: ethyl acetate:methanol:acetic acid, 9:3:2. Chromatographic purification was carried out by flash column chromatography using 40–63 μ silica gel (Merck) as the stationery phase.

Experimental details for the synthesis of proline *N*-benzhydrylglycolamide ester hydrochloride (**4**), N^α-(*tert*-butyloxycarbonyl)-glutaminyl-proline *N*-benzhydrylglycolamide ester (**6**), N^α-(*tert*-butyloxycarbonyl)-glutaminyl-prolinamide (**7**), pyroglutamyl-glutaminyl-prolinamide (**10**), N^α-(*tert*-butyloxycarbonyl)-glutamine *N*-benzhydrylglycolamide ester (**12**), and pyroglutamyl-glutamine *N*-benzhydrylglycolamide ester (**13**) have been previously published.[41]

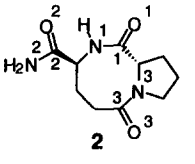
(3*S*,8a*S*)-3-(2-Carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo[1,2-*a*]pyra-zin-1,4-dione (1) and pyroglutamyl-proline *N*-benzhydrylglycolamide ester (9). N^α-(*tert*-Butyloxycarbonyl)-glutaminyl-proline *N*-benzhydrylglycolamide ester (**6**, 1.69 g, 3

Table 8. Interatomic distances (Å) between atoms of amide moieties calculated for minimum-energy conformers of compounds 1 and 2



1

$D_{O^1-O^2}$	$D_{C^1-C^2}$	$D_{N^1-N^2}$	$D_{O^2-O^3}$	$D_{C^2-C^3}$	$D_{N^2-N^3}$	$D_{O^1-O^3}$	$D_{C^1-C^3}$	$D_{N^1-N^3}$
6.32	5.42	5.39	5.40	4.99	7.12	5.33	2.93	2.79



2

$D_{O^1-O^2}$	$D_{C^1-C^2}$	$D_{N^1-N^2}$	$D_{O^2-O^3}$	$D_{C^2-C^3}$	$D_{N^2-N^3}$	$D_{O^1-O^3}$	$D_{C^1-C^3}$	$D_{N^1-N^3}$
3.73	3.05	3.44	7.00	4.79	5.48	4.89	3.02	2.79

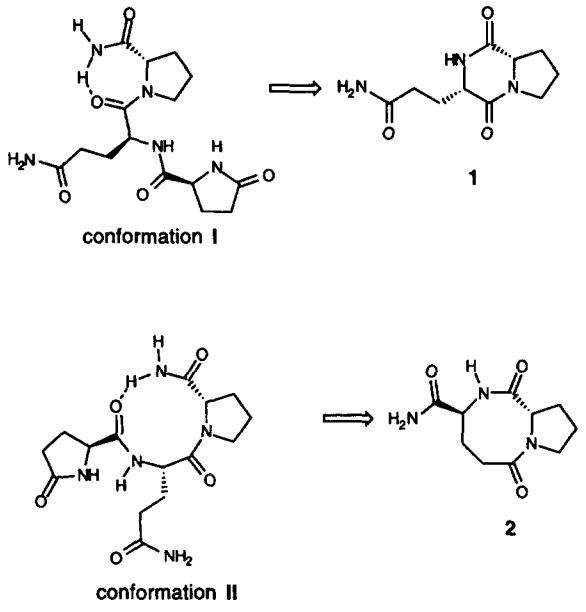


Figure 3. Transition from quasi-cyclic conformations to their cyclic mimetics.

mmol) was treated with 4 N HCl–dioxane (20 mL) for 30 min at room temperature. After removal of the solvent by vacuum, the residue was dried over potassium hydroxide pellets and dissolved in DMF (10 mL). *i*-Pr_2NEt (0.5 mL, 3 mmol) was added to the solution. The mixture was stirred at ambient temperature overnight and filtered. The filtrate was diluted with ether and the isolated oil was triturated with methanol to yield (3*S*,8a*S*)-3-(2-carbamoylethyl)-2,3,6,7,8,8a-hexahydro-1*H*,4*H*-pyrrolo[1,2-*a*]pyrazin-1,4-dione (**1**). The methanolic filtrate was concentrated and the residue was purified by flash chromatography, using EtOAc and EtOAc:MeOH (9:1) in consecutive order to obtain pyroglutamylproline *N*-benzhydrylglycolamide ester (**9**) and an additional amount of **1**. The total yield of **1** was 0.4 g (60%); mp 238–241°C; R_f 0.25 (C); $[\alpha]^{20}_{578}$ −122.5 (*c* 1; MeOH); ^{1}H NMR δ 8.20 (s, 1, NH), 7.33, 6.86 (ss, 2, NH_2), 4.21 (m, 1, Gln-αCH), 4.10 (t, *J* = 5.0, 1, Pro-CH), 3.50–3.35 (m, 2, Pro-δCH_2), 2.40–2.10 (m, 3, Pro-βCH and Gln-γCH_2), 2.10–1.75 (m, 5, Pro-γCH_2, Pro-βCH, Gln-βCH_2). **9**: yield 0.2 g (15%); mp 200–202 °C; $[\alpha]^{20}_{578}$ −58.6° (*c* 0.5; MeOH); ^{1}H NMR δ 8.86 (d, *J* = 8.5 Hz, 1, N*H*CH), 7.86 (s, 1, Glp-NH), 7.45–7.25 (m, 10, aromatic), 6.17 (d, *J* = 8.4 Hz, 1, NHC*H*), 4.69 (s, 2, CH_2CO), 4.45 (m, 2, Pro-αCH, Glp-αCH), 3.80–3.60 (m, 1, Pro-δCH), 3.60–3.45 (m, 1, Pro-CH), 2.50–1.80 (m, 6, Glp-βCH_2, Pro-βCH_2, Pro-CH_2).

N^{α}-(*tert*-Butyloxycarbonyl)-glutamic acid α-(*N*-benzhydrylglycolamide) ester (15). N^{α}-(*tert*-Butyloxycarbonyl)-glutamic acid (1.73 g, 7 mmol) was dissolved in anhydrous methanol (2 mL), 1.8 M CH_3ONa (3.9 mL) and 15-crown-5 (1.4 mL, 7 mmol) were added to the solution. After evaporation of methanol, a solution of *N*-(diphenylmethyl)bromoacetamide (2.13 g, 7 mmol) in ethyl acetate (30 mL) was added to the residue. The reaction solution was stirred for 3 h at 50 °C and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in a vacuum to yield 2.4 g (73%) of **15**; ^{1}H NMR δ 8.89 (d, 1, N*H*CH), 7.43 (d, 1, Glu-NH), 7.41–7.27 (m, 10, $(C_6H_5)_2$), 6.18 (d, 1, $(C_6H_5)_2CH$), 4.70 (m, 2, CH_2CO), 4.07 (m, 1, Glu-CH), 2.34 (m, 2, Glu-γCH_2), 2.03 (m, 1, Glu-βCH), 1.81 (m, 1, Glu-βCH), 1.40 (s, 9, Boc-CH_3).

N^{α}-(*tert*-Butyloxycarbonyl)-glutamyl[α-(*N*-benzhydrylglycolamide) ester]-proline (16). The mixture of N^{α}-(*tert*-butyloxycarbonyl)-glutamic acid α-(*N*-benzhydrylglycol-amide) ester (**15**, 1.41 g, 3 mmol), *N*-hydroxysuccinimide (0.38 g, 3.3 mmol), and DCC (0.68 g, 3.3 mmol) in tetrahydrofuran was stirred at room temperature overnight. The precipitate was filtered off, and the filtrate was concentrated in vacuum to afford N^{α}-(*tert*-butyloxycarbonyl)-glutamic acid α-(*N*-benzhydrylglycolamide) ester γ-(*N*-hydroxysuccin-imide) ester. Proline (0.38 g, 3.3 mmol) was dissolved in methanol containing 1.83 mL of 1.8 M CH_3ONa and 15-crown-5 (0.7 mL). The solution was concentrated. The complex formed was dissolved in 15 mL of DMF and added to N^{α}-(*tert*-butyloxycarbonyl)-glutamic acid α-(*N*-benzhydrylglycolamide) ester γ-(*N*-hydroxysuccinimide) ester. The reaction mixture was stirred overnight at room temperature and diluted with ethyl acetate (30 mL), acetic acid (0.3 mL), and water (30 mL). The aqueous layer was extracted with ethyl acetate (30 mL). The combined organic layers were washed with 10% citric acid, brine, dried over $MgSO_4$, and concentrated in a vacuum. The residue was chromatographed (acetone:hexane, 1:1) to yield 1.27 g (73.3%) of N^{α}-(*tert*-butyloxycarbonyl)-glutamyl[α-(*N*-benzhydrylglycolamide)ester]-proline (**16**) as oil; R_f 0.33 (A), 0.52 (D); $[\alpha]^{20}_{578}$ −3.5° (*c* 0.5; MeOH); ^{1}H NMR δ 12.50 (bs, 1, COOH), 8.90 (d, 1, N*H*CH), 7.46 (d, 1, Glu-NH), 7.41–7.27 (m, 10, $(C_6H_5)_2$), 6.18 (d, 1, $(C_6H_5)_2CH$), 4.70 (m, 2, CH_2CO), 4.25 (m, 1, Pro-CH), 4.09 (m, 1, Glu-αCH), 3.48 (m, 2, Pro-δCH_2), 2.30–1.80 (m, 8, Glu-γCH_2, Glu-βCH_2, Pro-βCH_2, Pro-CH_2), 1.40 (s, 9, Boc-CH_3).

(8*S*,10a*S*)-8-[*N*-(Diphenylmethyl)carbamoylmethoxycarbonyl]-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (17). N^{α}-(*tert*-Butyloxycarbonyl)-glutamyl[α-(*N*-benzhydrylglycolamide) ester]-proline (**16**, 1.135 g, 2 mmol) was dissolved in tetrahydrofuran (15 mL). *N*-Hydroxysuccinimide (0.253 g, 2.2 mmol) and DCC (0.45 g, 2.2 mmol) were added to the solution. The reaction mixture was stirred for 3 h at room temperature and filtered. The filtrate was evaporated and the residue was treated with 4 N HCl/dioxane (6 mL) for 30 min. The solvent was removed in vacuum. The residue was dried over KOH pellets in a vacuum and dissolved in acetonitrile (150 mL). Triethylamine (0.3 mL) in acetonitrile (10 mL) was added to the solution for 30 min. The mixture was stirred for 3 h at room temperature and concentrated in a vacuum. A solution of the residue

in ethyl acetate (30 mL) was washed with 1 N HCl, brine, 5% $NaHCO_3$ and brine consequently. The organic layer was dried over $MgSO_4$ and concentrated in a vacuum. The residue was triturated with ether to give 0.667 g (71.5%) of (8*S*,10a*S*)-8-[*N*-(diphenylmethyl)carbamoylmethoxycarbonyl]-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (**17**); mp 116–118 °C; $[\alpha]^{20}_{578}$ −6.8° (*c* 0.5; MeOH); R_f 0.46 (D); ^{1}H NMR δ 9.13 (d, 1, N*H*CH), 7.40 (d, 1, Glu-NH), 7.42–7.27 (m, 10, $(C_6H_5)_2$), 6.18 (d, 1, $(C_6H_5)_2CH$), 4.81 (m, 2, CH_2CO), 4.57 (m, 1, Pro-αCH), 4.42 (m, 1, Glu-αCH), 3.53 (m, 1, Pro-CH), 3.22 (m, 1, Pro-δCH), 2.50–1.80 (m, 8, Glu-CH_2, Glu-βCH_2, Pro-βCH_2, Pro-γCH_2).

(8*S*,10a*S*)-8-Carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (2). (8*S*,10a*S*)-8-[*N*-(Diphenylmethyl)carbamoylmethoxycarbonyl]-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo-[1,2-*a*][1,4]diazocin-5,10-dione (**17**, 0.653 g, 1.4 mmol) was dissolved in dioxane (10 mL) saturated with NH_3. The solution was left for 3 days and evaporated. The residue was purified by flash chromatography ($CHCl_3$:CH_3OH, 4:1) to yield 0.244 g (70.5%) of (8*S*,10a*S*)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5*H*,10*H*-pyrrolo[1,2-*a*][1,4]diazocin-5,10-dione (**2**); mp 232–234 C; $[\alpha]^{20}_{578}$ −22.2° (*c* 0.5; MeOH); R_f 0.29 (B); ^{1}H NMR δ 7.88, 7.46 (ss, 2, $CONH_2$), 6.78 (bs, 1, Glu-NH), 4.63 (t, 1, Pro-αCH), 4.15 (m, 1, Glu-αCH), 3.66 (m, 1, Pro-δCH), 3.35 (m, 1, Pro-δCH), 2.65 (m, 1, Glu-γCH), 2.59 (m, 1, Pro-CH), 2.29 (m, 1, Pro-βCH), 2.08 (m, 1, Glu-βCH), 1.83 (m, 1, Glu-βCH), 1.81 (m, 2, Pro-γCH_2), 1.80 (m, 1, Glu-γCH).

Pharmacology

Experiments were carried out on white unbred rats (170–230 g) after intraperitoneal administration. Injections were performed 10 min before experiments, except for EEG. Differences from the control were assessed statistically using ANOVA.

Amnesia-reversal testing. Two different amnesic models were chosen to investigate a general protective effect on the CNS against electroconvulsive shock (ECS) treatment[63] and an effect related to the cholinergic system against scopolamine administration[58] upon the passive avoidance response. Scopolamine hydrobromide was administered intraperitoneally in a dose of 2.5 mg/kg 15 min before the training. Twenty-four hours after the acquisition trial, the effects of the compounds were evaluated by the level of retention of the required response, namely to avoid entering the dark compartment over a period of 120 s. The antiamnesic effect was calculated using the following formula:[64]

$$\text{amnesia reversal } (\%) = 100 \times ((t_3 - t_2)/(t_1 - t_2)$$

where t_1 is retention latency (time taken to enter the dark compartment) for the sham group which were not treated by the amnesic agents ECS nor Scopolamine, where t_2 = retention latency for the control group which were injected with saline and received ECS or Scoplamine and t_3 = retention latency for the experimental group which were injected with substances and received amnesic treatment.

Antidepressant activity. This was studied in the following modification of a forced swimming test.[52] Animals were placed individually into a glass beaker (diameter 30 cm, height 50 cm) that was filled with water (19–21 °C) to two-thirds of its volume. Rats were individually forced to swim inside the beaker, and the effect of the compounds was determined by measuring the immobility time of animals during a 5-min period. The registration began immediately after location of animals into the cylinder. The rats were judged to be immobile whenever they remained floating passively with their heads just above the water. The antidepressant pyrazidol (8-methyl-2,3,3a,4,5,6-hexahydro-1*H*-pyrido[3,2,1-*j*,*k*]carbazol) was applied as the reference compound.

Antihypoxic effect. This was studied using the model of acute hypobaric hypoxia in a flowthrough pressure chamber model.[65] The animals in the experimental group (16 rats) were administered intraperitoneally compound **2** 10 min before the experiment. The animals in the control group (16 rats) were injected with saline. Groups of four animals were lifted with the speed 1 km/min to the platform with the height of 11 km. Animals were kept on this platform for 15 min and then put down with the speed 3.7 km/min. Ascent to the high altitude was simulated experimentally by exposure of rats in a chamber to air at an alterable pressure. Fifty-six percent of the animals survived in the experimental group in comparison with 18% in the control group.

To detect the neurological deficit, myorelaxant and sedative action, motor impairment and psychomotor activation were investigated in the rotarod test,[66] the traction test[67] and the open field test.[68]

EEG evaluation. The effects on EEG were studied using methods of quantitative pharmacoencephalography.[60,69] One day before the experiment, two cortical electrodes were fitted, under ether narcosis, over the area postsentralis oralis (sensorimotor area) and area striata (visual cortex). A state of vigilance was induced by the method of forced continuous ambulation.[70] The EEG was transmitted by a shielded cable to EEGP4-02 electroencephalograph, which was interfaced to an Alpha-BK microcomputer. The microcomputer was applied for digital recording and processing of 30 s sampled EEG using the amplitude-interval analysis techniques.[71] The system of EEG parameters included 14 indices: total average frequency, total average amplitude and the average frequencies, amplitudes and time indexes for delta-, theta-, alpha-, and beta-bands of EEG. D^2

Mahalanobis statistic was served as an integral index of drug effect on EEG. Mahalanobis distances were calculated for the total 30 s sampled EEG sums for the control and experimental groups during intervals of time 0–30 min, 30–60 min, and 60–90 min after administration. Mahalanobis distance reflects the difference between EEG of control and experimental groups of animals.

References

1. For a recent review of TRH, see O'Leary, R.; O'Connor, B. *J. Neurochem.* **1995**, *65*, 953.

2. Bler, J.; Enzmann, F.; Folkers, K.; Bowers, C. Y.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 705.

3. Burgus, R.; Dunn, T. F.; Desiderio, D.; Vale, W.; Guillemin, R. *C. R. Acad. Sci. (Paris)* **1969**, *269*, 1870.

4. Metcalf, G.; Dettmar, P. W. *Lancet* **1981**, *1*, 586.

5. (a) Breese, G. R.; Cott, J. M.; Cooper, B. R.; Prange, A. J.; Lipton, M. A. *Life Sci.* **1974**, *14*, 1053. (b) Breese, G. R.; Cott, J. M.; Cooper, B. R.; Prange, A. J.; Lipton, M. A.; Plotnikoff, N. P. *J. Pharmacol. Exp. Ther.* **1975**, *193*, 11. (c) Wei, E.; Sigel, S.; Loh, H.; Way, E. L. *Nature (London)* **1975**, *253*, 739.

6. (a) Yarbrough, G. *Nature (London)* **1976**, *263*, 523. (b) Yarbrough, G. *Life Sci.* **1983**, *33*, 111. (c) Horita, A.; Carino, M. A.; Lai, H. *Annu. Rev. Pharmacol. Toxicol.* **1986**, *26*, 311. (d) Okada, M. *J. Neurochem.* **1991**, *56*, 1544. (e) Stwertka, S. A.; Vincent, G. P.; Gamzu, E. R.; MacNeil, D. A.; Vederese, A. *Pharmacol. Biochem. Behav.* **1991**, *41*, 145.

7. Griffiths, E. C. *Clin. Sci.* **1987**, *73*, 449.

8. *Thyrotropin-Releasing Hormone*; Griffiths, E. C.; Bennett, J. R., Eds.; Raven: New York, 1983.

9. Yarbrough, G.; Pomara, M. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1985**, *9*, 285.

10. Albert, M.; Jenike, M.; Nixon, R.; Nobel, K. *Biol. Psychiatry* **1993**, *33*, 267.

11. Tsuboyama, G. K.; Gabriel, S. S.; Davis, B. M.; Davidson, M.; Lawlor, B. A.; Ware, K.; Davis, K. L.; Mohs, R. S. *Biol. Psychiatry* **1993**, *32*, 195.

12. Lafer, B.; Fava, M.; Hammerness, P.; Rosenbaum, J. F. *Biol. Psychiatry* **1993**, *34*, 650.

13. Maeda, K.; Yoshimoto, Y.; Yamadori, A. *Biol. Psychiatry* **1993**, *33*, 277.

14. Nemeroff, C. B.; Widerlov, E.; Bissette, G.; Walleus, H.; Karlsson, I.; Ekluno, K.; Kilts, C. D.; Loosen, P. T.; Vale, W. *Science* **1984**, *226*, 1342.

15. Renming, X.; Ishihara, K.; Sasa, M.; Ujihara, H.; Momiyama, T.; Fujita, Y.; Todo, N.; Serikawa, T.; Yamada, J.; Takaori, S. *Eur. J. Pharmacol.* **1992**, *223*, 185.

16. Kubek, M. J.; Knoblach, S. M.; Sharif, N. A.; Burt, D. R.; Buterbaugh, G. G.; Fuson, K. S. *Ann. Neurol.* **1993**, *33*, 70.

17. Duthie, S. M.; Taylor, P. L.; Anderson, L.; Cook, J.; Eidne, K. A. *Mol. Cell. Endocrinol.* **1993**, *95*, R11.

18. Alamo, C.; Vallejo, M.; Cuenca, E. *Arch. Pharmacol. Toxicol.* **1982**, *8*, 151.

19. Faakkari, P.; Feuerstein, G. *Neuropharmacology* **1988**, *27*, 1007.

20. Sharif, N. A.; To, Z.; Whiting, R. L. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 1306.

21. McDermott, A. M.; Dickinson, S. L.; Wilkin, G. P. *Neurochem. Int.* **1992**, *20*, 307.

22. Olson, G. L.; Cheung, H.-Ch.; Chiang, E.; Madison, V. S.; Sepinwall, J.; Vincent, G. P.; Winokur, A.; Gary, K. A. *J. Med. Chem.* **1995**, *38*, 2866.

23. Miyamoto, M.; Yamazaki, N.; Nagaoka, A.; Nigawa, Y. *Ann. N.Y. Acad. Sci.* **1989**, 508.

24. Vonhof, S.; Feuerstein, G. Z.; Cohen, L. A.; Labroo, V. M. *Eur. J. Pharmacol.* **1990**, *180*, 1.

25. Crick, F. H. C. *J. Mol. Biol.* **1966**, *19*, 548.

26. Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449.

27. Potts, Jr., J. T. In *Peptides Hormones*; Parsons, J. A., Ed.; University Park: Baltimore, 1976; p 134.

28. (a) Guillemin, R.; Brazeau, P.; Böhlen, P.; Esch, F.; Ling, N.; Wehrenberg, W. B. *Science* **1982**, *218*, 585. (b) Spiess, J.; Rivier, J.; Vale, W. *Nature (London)* **1983**, *303*, 532.

29. Schröder, E.; Lübke, K. *The Peptides*, Vol. II; Academic: New York, 1966; pp 52–53.

30. (a) Mazurov, A. A.; Andronati, S. A.; Kabanov, V. M.; Sokolenko, N. I.; Rokachinskaya, M. G.; Shapiro, Yu. E.; Gorbatyuk, V. Ya. *Collect. Czech. Chem. Commun.* **1990**, *55*, 2555. (b) Rokachinskaya, M. G.; Golovenko, N. Ya.; Kabanov, V. M.; Mazurov, A. A.; Andronati, S. A. *Physiologically Active Compounds (Kiev)* **1991**, *23*, 46.

31. Shapiro, Yu. E.; Gorbatyuk, V. Ya.; Kabanov, V. M.; Mazurov, A. A.; Andronati, S. A.; Lobasyuk, B. A.; Golovenko, N. Ya.; Rokachinskaya, M. G. *Sov. J. Bioorg. Chem.* **1990**, *16*, 1607.

32. Mazurov, A. A.; Andronati, S. A.; Lobasyuk, B. A.; Kabanov, V. M.; Korotenko, T. I. *Pharm. Chem. J.* **1988**, 155.

33. Kabanov, V. M.; Mazurov, A. A.; Andronati, S. A. *Sov. J. Bioorg. Chem.* **1992**, *18*, 1013, and references therein.

34. Endogenous pyroglutamyl-glutaminyl-prolinamide have been isolated from human seminal fluid: Khan, Z.; Aitken, A.; del Rio Garcia, J.; Smyth, D. G. *J. Biol. Chem.* **1992**, *267*, 7464.

35. Deslauriers, R.; Garrigou-Lagrange, Ch.; Bellocq, A.-M.; Smith, I. C. P. *FEBS Lett.* **1973**, *31*, 59.

36. (a) Montagut, M.; Lemanceau, B.; Bellocq, A.-M. *Biopolymers* **1974**, *13*, 2615. (b) Donzel, B.; Rivier, J.; Goodman, M. *Biopolymers* **1974**, *13*, 2631.

37. Gorbatyuk, V. Ya.; Shapiro, Yu. E.; Mazurov, A. A.; Zhuravlyov, V. G.; Andronati, S. A.; Korotenko, T. I.; Romanovsky, P. Ya. *Sov. J. Bioorg. Chem.* **1992**, *18*, 235.

38. Burket, U.; Allinger, N. L. *Molecular Mechanics*, American Chemical Society: Washington, D.C., 1982.

39. Peterson, R. E.; Guillemin, R. G. *Amer. J. Med.* **1974**, *57*, 591.

40. Amblard, M.; Rodriguez, M.; Martinez, J. *Tetrahedron* **1988**, *44*, 5101.

41. Mazurov, A. A.; Andronati, S. A.; Korotenko, T. I.; Gorbatyuk, V. Y.; Shapiro, Y. E. *Int. J. Peptide Protein Res.* **1993**, *42*, 14.

42. Mazurov, A.A.; Antonenko, S. V.; Andronati, S. A. *Chemistry of Natural Compounds (USSR)* **1986**, 208.

43. (a) Andronati, S. A.; Mazurov, A. A. *Sov. J. Bioorg. Chem.* **1984**, *10*, 1445. (b) Andronati, S. A.; Mazurov, A.A.; Korotenko, T. I. *Chemistry of Peptides and Proteins* **1986**, *3*, 37.

44. Kastin, A. J.; Ehrensing, R. H.; Schalch, D. S.; Anderson, M. S. *Lancet* **1972**, *2*, 740.

45. Prange, A. J., Jr.; Wilson, I. C.; Lala, P. P.; Alltop, L. B.; Breese, G. R. *Lancet* **1972**, *2*, 999.

46. Coppen, A.; Montgomery, S.; Peet, M.; Bailey, J. *Lancet* **1974**, *2*, 433.

47. Ehrensing, R. H.; Kastin, A. J.; Schalch, D. S.; Friesen, H. G.; Vagas, J. R.; Schally, A. V. *Am. J. Psychiatry* **1974**, *131*, 714.

48. Montjoy, C. O.; Price, J. S.; Weller, M.; Hunter, P.; Hall, R.; Dewar, J. D. *Lancet* **1974**, *1*, 958.

49. Ogawa, N.; Yamawaki, Y.; Kuroda, H.; Ofuji, T.; Itoga, E.; Kito, S. *Brain Res.* **1981**, *205*, 169.

50. Ogawa, N.; Yamawaki, Y.; Kuroda, H.; Nukina, I,; Ota, Z.; Fujino, M.; Yanaihara, N. *Peptides* **1982**, *3*, 669.

51. Ogawa, N.; Mizuno, S.; Mori, A.; Nukina, I.; Ota, Z.; Yamamoto, M. *Peptides* **1982**, *3*, 743.

52. Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. *Eur. J. Pharmacol.* **1978**, *47*, 379.

53. Schindler, U. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1989**, *13 (Suppl.)*, 99.

54. Banfi, S.; Dorigotti, L. *Clin. Neuropharmacol.* **1986**, *9 (Suppl. 3)*, S19.

55. Banfi, S.; Cornelli, U.; Fonio, W.; Dorigotti, L. *J. Pharmacol. Methods* **1982**, *8*, 255.

56. Pozzi, O.; Allievi, E.; Biagetti, R.; Banfi, S.; Dorigotti, L. *Pharmacol. Res. Commun.* **1988**, *20 (Suppl. 2)*, 315.

57. Schindler, U.; Rush, D. K.; Fielding, S. *Drug Dev. Res.* **1984**, *4*, 567.

58. Verloes, R.; Scotto, A. M.; Gobert, J.; Wlfert, E. *Psychopharmacology* **1988**, *95*, 230.

(*Received in U.S.A.* 9 *April* 1997; *accepted* 10 *June* 1997)

59. Pinza, M.; Farina, C.; Cerri, A.; Pfeiffer, U.; Riccaboni, M. T.; Banfi, S.; Biagetti, R.; Pozzi, O.; Magnani, M.; Dorigotti, L. *J. Med. Chem.* **1993**, *36*, 4214.

60. Fink, M. *Quantitative EEG Analysis and Psychopharmacology*; Elsevier: Amsterdam, 1977.

61. Cosentino, U.; Moro, G.; Pitea, D.; Todeschini, R.; Brossa, S.; Gualandi, F.; Scolastico, C.; Giannesi, F. *Quant. Struct.-Act. Relat.* **1990**, *9*, 195.

62. Altomare, C.; Cellamare, S.; Carotti, A.; Casini, G.; Ferappi, M.; Gavuzzo, E.; Mazza, F.; Carrupt, P.-A.; Gaillard, P.; Testa, B. *J. Med. Chem.* **1995**, *38*, 170.

63. Martinez, J. L., Jr.; Jensen, R. A.; McCaugh, J. L. *Prog. Neurobiol.* **1981**, *16*, 155.

64. Butler, D. E.; Nordin, I. C.; L'Italien, Y. L. *J. Med. Chem.* **1984**, *27*, 684.

65. Korablyov, M. V.; Lukienko, P. I. *Antihypoxic Compounds*; Minsk, 1976.

66. Dunham, N. W.; Miya, T. S. *J. Amer. Pharm. Assoc.* **1957**, *46*, 208.

67. Boissier, J.-R.; Dumont, C.; Ratouis, R.; Pagny, J. *Arch. Int. Pharmacodyn.* **1961**, *133*, 29.

68. Boissier, J.-R.; Simon, P. *Thérapie* **1962**, *17*, 1225.

69. *EEG in Drug Research*; Herrmann, W. M., Ed.; Stuttgart: New York, 1982.

70. Glatt, A.; Duerst, T.; Mueller, B.; Demieville, H. *Neuropsychobiology* **1983**, *9*, 163.

71. Sharp, F. H.; Smith, G. W. *Psychophysiology* **1975**, *12*, 471.