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DESIGN OF A NOVEL COGNITIVE ENHANCER (8S, 10aS)-8-CARBAMOYL-1,2,3,6,7,8,9,10a-OCTAHYDRO-5H,10H-PYRROLO[1,2-a][1,4]DIAZOCIN-5,10-DIONE

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Abstract. A new cognitive enhancer (8S, 10aS)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5H,10H-pyrrolo[1,2-a][1,4]diazocin-5,10-dione (**3**) has been designed based on the approach consisting of two steps: 1) "obligatory" replacement of histidine with glutamine in TRH; 2) the application of conformational constraints for putative bioactive conformation of [Gln^2]-TRH. **3** reversed on 100% electroconvulsive shock-induced amnesia at doses of 0.1 and 1.0 mg/kg and on 83% scopolamine-induced amnesia at a dose of 1 mg/kg, using passive avoidance test.

Thyrotropin-releasing hormone (TRH, Glp-His-Pro-NH_2) like many of peptide hormones provoke a wide range of biological responses. In addition to governing the secretion of pituitary hormones such as thyroid stimulating hormone (TSH, thyrotropin) and prolactin, TRH has been characterized as CNS-activating substance functioning either as a neurotransmitter or as a facilitative neuromodulator[1]. In particularly, TRH facilitates cholinergic and monoaminergic neurotransmission[2] independently of its hormonal effect[3]. The established cholinergic deficit and resulting cholinergic treatment strategy in Alzheimer's disease and the ability of TRH and its analogs to enhance cognitive performance in behavioral models in animals[4] suggests, that TRH analogs represent a logical approach to treat cognitive disorders, including those associated with Alzheimer's disease.

For the purpose of rational modification of TRH molecule, we were pursuing an approach based on our assumption about the existence of "obligatory" similar amino acids. As "obligatory" similar amino acids, we considered pairs of amino acids encoded by the same obligatory nucleotides (Table 1). According to the Crick's "wobble hypothesis", the first two codone bases (obligatory nucleotides) make the most significant contribution into the specific encoding in comparison with the third base (facultative nucleotide)[5]. From twenty 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 can replace each other with at least preservation of some kinds of biological effects in certain examples. Indeed, there are some natural

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occurring peptides with similar biological potency and those peptides contain "obligatory" similar amino acids, for example, magainins[6], growth hormone-releasing factor[7], calcitonins[8], angiotensin analogues[9].

Table 1. "Obligatory" Similarity of Amino Acids

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

Earlier, the application of the hypothesis of "obligatory" similar amino acids has been demonstrated for neurohormone MSH release inhibiting factor (Pro-Leu-Gly-NH_2, MIF, melanostatin)[10,11]. According to the above mentioned hypothesis, the second amino acid of melanostatin leucine was exchanged with phenylalanine. Both of the peptides MIF and [Phe^2]-MIF showed similar profile of locomotor activity after intracerebroventricular administration[12]. The level of major neurotropic effect of MIF, antidepressant activity, was approximately retained after obligatory replacement of leucine[10].

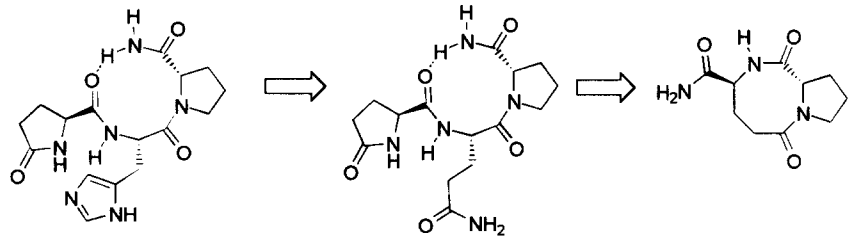
Analogous "obligatory" replacement of second amino acid histidine by glutamine were undertaken for thyroliberin. Both TRH (**1**) and [Gln^2]-TRH (**2**) have great conformational similarities (Table 2).

Table 2. Dihedral angles (deg) for amino acid residues in Glp-His-Pro-NH_2 (**1**) and Glp-Gln-Pro-NH_2 (**2**) according to NOESY data.

	ϕ_1	ψ_1	ϕ_2	ψ_2	$\chi^1{}_2$	$\chi^2{}_2$	ϕ_3	ψ_3
1	120	-30;180	-89;-152	0;170	±125	0;180	-	0
2	120	60;160	-88;152	120	±126	±60;180	-	0

ϕ, ψ, χ^1, χ^2 - dihedral angles around N-C$^\alpha$, C$^\alpha$-CO, C$^\alpha$-C$^\beta$ and C$^\beta$-C$^\gamma$ bonds for first, second and third amino acid residues, consequently

Conformations of TRH and Glp-Gln-Pro-NH_2 in $(CD_3)_2SO$ solution were determined using two-dimensional ^1H NMR spectroscopy (δ - J correlated, COSY, and NOESY) in accordance with approach applied for MIF and TRH analogues[11,13]. Earlier, 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 receptor[14]. 20% of [Gln²]-TRH molecules exist in quasicyclic conformation in solution. Peptide **2** retains antiamnesic activity of TRH[15].

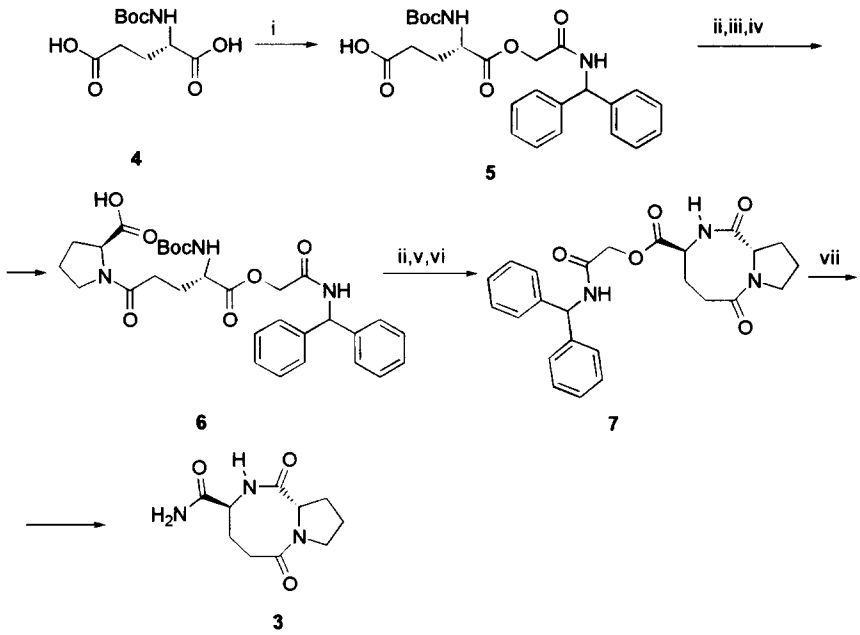


1 **2** **3**

Figure 1. Transition from putative bioactive conformation of TRH (**1**) via its "obligatory" similar analog **2** to mimetic **3**.

In order to stabilize the putative bioactive conformations of TRH and its "obligatory" similar analog Glp-Gln-Pro-NH_2 with intramolecular hydrogen bonds between C-terminal carboxamide proton and α-carbonyl of pyroglutamyl (Fig. 1), we synthesized mimetic **3** (Scheme 1). (8S, 10aS)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5H,10H-pyrrolo[1,2-a][1,4]diazocin-5,10-dione **3** has no pyrrolidone fragment, which is important pharmacophore feature for classical nootropic drugs like pyracetam and its analogues. Since histidine residue in TRH was replaced with "obligatory" similar glutamine, side chain moiety of glutamine was incorporated into designed compound **3**. Position of carbamoyl group in pyrrolodiazocine ring was determined based on distance between nitrogen atoms of glutamine side chain and proline in minimum-energy conformations of peptide **2**[16].

N-Boc-glutamic acid **4** forms with one equivalent of sodium methoxide in the presence of crown ether soluble in organic solvents complex that was converted into N-benzhydrylglycolamide ester **5** at mild conditions[17] using substantial difference in acidity of α- and γ-carboxyl moieties. Reaction of N-protected glutamic acid α-ester **5** with proline was performed by modified method of "salt condensation"[18]. Carboxyl function of proline was blocked by transformation into complex of proline sodium salt with 15-crown-5. Solution of the latter in N,N-dimethylformamide was treated by N-*tert*-butyloxycarbonyl glutamic acid α-benzhydrylglycolamide ester γ-(N-hydroxysuccinimide) ester to afford dipeptide **6** after neutralization of reaction mixture with acetic acid. In order to form eight-member ring, active ester method was applied. Glutamyl(α-benzhydrylglycolamide ester)-proline N-hydroxysuccinimide ester obtained after deprotection of amino moiety cyclized into pyrrolo[1,2-a][1,4]-diazocine **7**. Hydrophobic diphenylmethyl fragment facilitates the isolation and purification of the heterocycle. Meanwhile, benzhydrylglycolamide ester was readily converted into amide **3** by ammonolysis in dioxane to afford the desired compound.



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

Scheme 1.

(8S,10aS)-8-carbamoyl-1,2,3,6,7,8,9,10a-octahydro-5H,10H-pyrrolo[1,2a][1,4]-diazocin-5,10-dione (**3**) was tested for anti-depressant and nooropic effects after intraperitoneal administration. Anti-depressant potency was examined in experimental model of behavioral despair[19]. Cognition enhancing activity (nooropic effect) was assessed by passive avoidance test[20,21] in which amnesia was induced in rats by electroconvulsive shock (ECS) or scopolamine. Aniracetam, which is considered to be one of major enhancers of cognitive functions, was applied as a reference compound.

Results of forced swimming test indicate the absence of anti-depressive activity for pyrrolo[1,2-a][1,4]-diazocine **3**[22]. Meanwhile, significant antiamnesic potency was discovered in the passive avoidance test for this compound. It reversed completely ECS induced amnesia at doses 0.1 ($p<0.05$) and 1.0 mg/kg ($p<0.05$). Since amnesic effect of scopolamine have been attributed to a central cholinergic action[23,24] and cholinergic impairment has associated with the aging of the brain and senile dementia, we evaluated the ability of diazocine **3** 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% ($p<0.01$) at dose 1 mg/kg whereas oxiracetam[25] shows

only a 12% reversal of this deficit at the same dose. In the acute hypobaric hypoxia model[26], the compound showed antihypoxic activity in the dose of 0.1 mg/kg ($p<0.05$). The new compounds in neuropharmacological tests[27] in mice had no sedative and myorelaxant action, motor impairment and psychomotor activation on CNS.

The obtained results with "obligatory" replacement of amino acids in small biologically active peptide TRH forming quasicyclic conformation, which are stabilized by hydrogen bond, demonstrate the application of proposed approach for purposeful design of new biologically active compounds.

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