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A Study of the Biologically Active Conformation of the Cholecystokinin-4 Dipeptide Analogue GB-115

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Abstract—The biologically active conformation of *N*-(6-phenylhexanoyl)glycyl-tryptophan amide (GB-115), a highly active cholecystokinin-4 retro dipeptide analogue with the anxiolytic activity, has been studied using the conformational analysis by ^{1}H NMR spectroscopy in solution and the method of sterically restricted analogues. A study of the relationship between the preferable conformation in solution and the anxiolytic activity in the series of GB-115 derivatives showed that the biologically active conformation of this compound is the β-turn. Based on the data on the nuclear Overhauser effect ^{1}H NMR spectroscopy, this structure was identified as the β-turn of type II. Subsequent synthesis and study of the pharmacological activity of novel sterically restricted analogues of dipeptide GB-115: (2*S*)-2-{(3*R*)-3-[(6-phenylhexanoyl)amino]-2-oxopyrrolidine-1-yl}-3-(1*H*-indole-3-yl)propionic acid ethyl ester, *N*-(6-phenylhexanoyl)glycyl-N^{α}-methyltryptophan ethyl ester, (2*S*)-2-[(10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-ylcarbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester, and (2*S*)-2-[({3-[(ethoxycarbonyl)amino]-10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-yl}carbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester confirmed that the β-turn of type II is the active conformation of GB-115.

Keywords: cholecystokinin-4, dipeptide analogue, GB-115, ^{1}H NMR spectroscopy, biologically active conformation, sterically restricted analogues, conformational analysis

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INTRODUCTION

On the basis of the native anxiogenic tetrapeptide cholecystokinin-4 (Trp-Met-Asp-Phe-NH_2), its retro dipeptide analogue *N*-(6-phenylhexanoyl)glycyl-tryptophan amide was constructed in the Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences, using the topochemical principle of Shemyakin–Ovchinnikov–Ivanov [1] [2]. In animal models, this dipeptide at doses of 0.0025–0.25 mg/kg intraperitoneally and 0.1–0.8 mg/kg orally exhibits anxiolytic activity, has no by-effects typical for tranquilizers of the benzodiazepine series, and is almost nontoxic (LD_{50} > 6 g/kg for rats per os) [3, 4]. GB-115 can be a progenitor of a new group of anxiolytics with the cholecystokinin mechanism of action. In this connection, the question concerning the biologically active conformation of GB-115 is of current interest.

In the present study, the biologically active conformation of GB-115 was examined using the conformational analysis by ^{1}H NMR spectroscopy in solution and the method of spatially restricted analogues.

RESULTS AND DISCUSSION

To determine the biologically active conformation of the dipeptide GB-115, we studied the relationship between the structure and the anxiolytic activity in the series of GB-115 analogues of both the previously described *N*-(6-phenylhexanoyl)prolyl-tryptophan amide (**I**) and *N*-(6-phenylhexanoyl)prolyl-tryptophan ethyl ester (**II**) [2, 5] and the novel conformationally mobile [*N*-(4-phenylbutyryl)glycyl-tryptophan methylamide (**III**), *N*-(5-phenylpentanoyl)glycyl-tryptophan methylamide (**IV**), and *N*-(6-phenylhexanoyl)prolyl-tryptophan metyhylamide (**V**)] and spatially restricted analogues: (2*S*)-2-{(3*R*)-3-[(6-phenylhexanoyl)amino]-2-oxopyrrolidine-1-yl}-3-(1*H*-indole-3-yl)propionic acid ethyl ester (**VI**), *N*-(6-phenylhexanoyl)glycyl-N^{α}-(methyl)-tryptophan ethyl ester (**VII**), (2*S*)-2-[(10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-ylcarbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester (**VIII**), and (2*S*)-2-[({3-[(ethoxycarbonyl)amino]-10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-yl}-carbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester (**IX**).

Abbreviations: EPM, elevated plus-maze; IHB, intramolecular hydrogen bond; NOE, nuclear Overhauser effect; the configuration symbol in amino acids of the *L* series is omitted; MeTrp, N^{α}-methyltryptophan.

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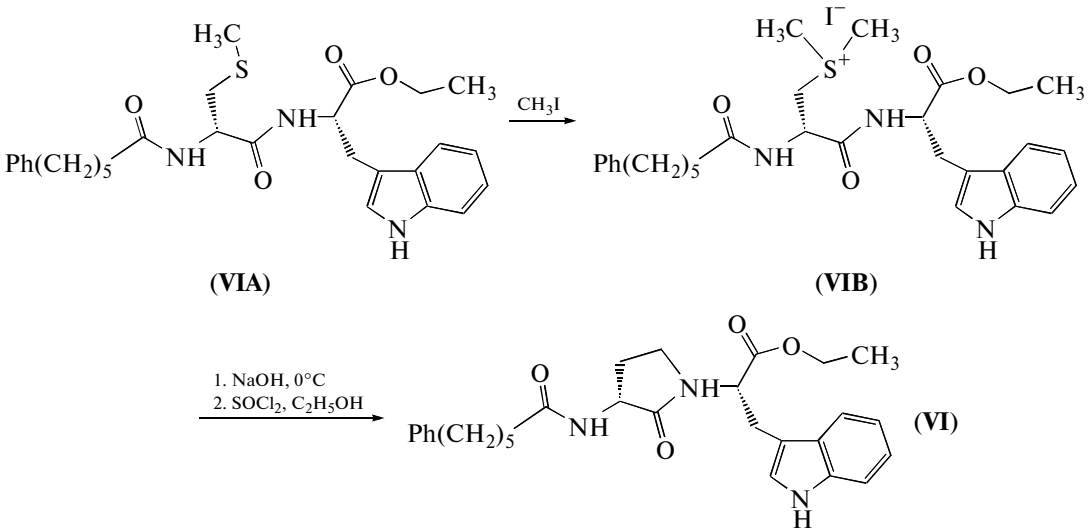
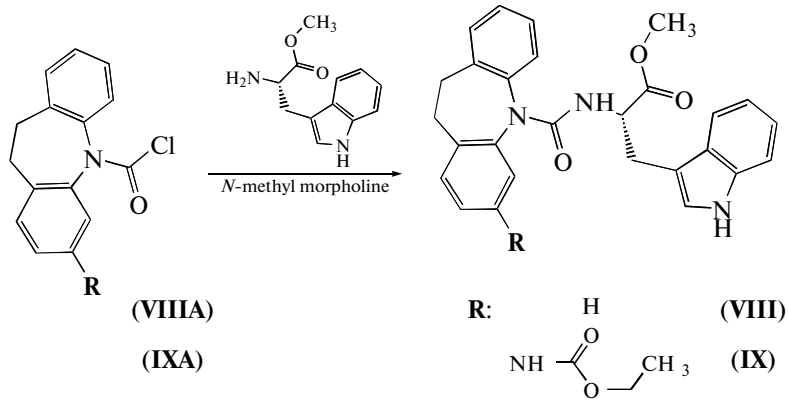
Substituted peptides were obtained by the method of mixed anhydrides as described earlier [2, 3, 5]. Compound (**VI**), which contains an aminolactam fragment, was synthesized by the method of Freidinger [6, 7] from *N*-(6-phenylhexanoyl)-*D*-methionyl-tryptophan ethyl ester (**VIA**), which was converted by means of methyl iodide to sulfonium salt and then cyclized to lactam in the presence of sodium hydride. Because ethyl ester was completely hydrolyzed under the conditions of cyclization, the resulting acid was esterified again by ethanol in the presence of thionyl chloride (Scheme 1).



(**VIA**) (**VIB**) (**VI**)

Scheme 1. Synthesis of the γ-lactam analogue (**VI**).

Both dibenzoazepine analogues (**VIII**) and (**IX**) were synthesized by acylating the methyl ester of tryptophan by the corresponding *N*-chlorocarbonyldibenzoazepine derivative (Scheme 2).



(**VIIIA**) (**VIII**)

(**IXA**) (**IX**)

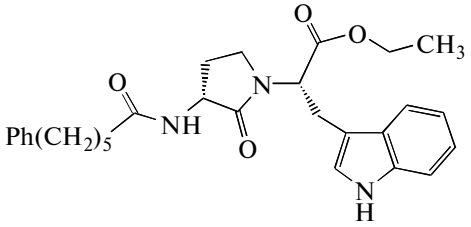
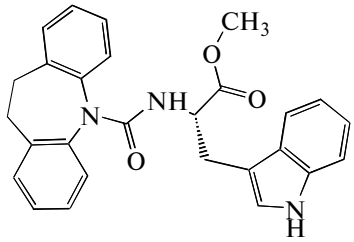
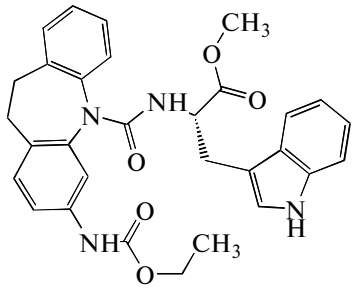
Scheme 2. Synthesis of dibenzoazepine analogues (**VIII**) and (**IX**).

The pharmacological activity of the synthesized compounds (Table 1) was studied using the EPM test, which is the major model for assessing the anxiolytic and anxiogenic activities [8].

It is known that the proline residue contributes to a greater extent to the formation of the turn conformation of the peptide chain than the glycine residue [9–11]. Because the transition of the glycine-containing GB-115 to its proline-containing analogue (I) is accompanied by an increase in activity, as is evident from Table 1, we concluded that the biologically active conformation of GB-115 is the turn.

To determine the type of the turn structure, the conformational analysis of compounds GB-115 and (**I**) in solution was carried out by ^{1}H NMR spectroscopy. One-dimensional ^{1}H NMR spectra and the spectra of ^{1}H-^{1}H double homonuclear resonance in DMSO-d_6 and $CDCl_3$ solutions were measured.

Table 1. Data of the pharmacological study of conformationally restricted analogues of GB-115 in the EPM test on mongrel rats

Compound		Dose, mg/kg ip (*n*)	Parameter	
Formula	Code or number		Runs into open arms, %	Time of staying in open arms, %
$Ph(CH_2)_5CO$-**Gly**-Trp-$\mathbf{NH_2}$	**GB-115**	0 (14) 0.01 (14) **0.05** (16) **0.1** (8) **0.2** (8)	13.0 27.2 34.1* 42.1* 31.0*	4.0 10.9 12.5* 13.0* 11.9*
$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{NH_2}$	**(I)**	0 (6) **0.01** (8) **0.05** (6) 0.1 (6) 0.5 (5)	25.9 47.7* 42.7* 37.9 40.7	10.6 30.4* 27.2* 20.1 20.5
$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{OC_2H_5}$	**(II)**	0 (6) 0.01 (6) 0.05 (7)	33.9 38.5 36.9	7.7 10.7 9.3
$Ph(CH_2)_3CO$-**Gly**-Trp-$\mathbf{NHCH_3}$	**(III)**	0 (8) 0.8 (8) 1.6 (8) 3.2 (8)	4.2 7.0 6.3 16.6	0.1 1.2 0.5 5.4
$Ph(CH_2)_4CO$-**Gly**-Trp-$\mathbf{NHCH_3}$	**(IV)**	0 (12) 0.1 (10)	18.4 6.4	6.7 2.5
$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{NHCH_3}$	**(V)**	0 (10) 0.01 (5) **0.05** (7)	25.3 35.2 39.3*	6.5 12.4 24.3*
	**(VI)**	0 (6) 0.01 (8) **0.05** (8)	32.7 35.8 53.5*	22.6 38.9 56.8*
$Ph(CH_2)_5CO$-**Gly**-MeTrp-$\mathbf{OC_2H_5}$	**(VII)**	0 (12) 0.01 (6) 0.05 (8)	25.8 28.2 36.5	15.1 12.0 23.8
	**(VIII)**	0 (6) 0.05 (6) 0.5 (6)	44.6 36.6 32.1	15.9 17.4 17.8
	**(IX)**	0 (6) **0.05** (6) 0.5 (6)	44.6 48.5* 35.3	15.9 51.5* 24.4

Note: *n*, the number of animals; $*p < 0.05$, statistical significance of the difference from the control according to the Mann–Whitney U-test; ip, intraperitoneally.

Table 2. Δδ values and percentage of β-turn conformation in a non-polar medium (0.027 M, 25°C)

Compound	Δδ, ppm					β-turn fraction, % **
	NH_{ind}	NH_{Trp}	NH_{Gly}	NH^{free}_{amide}	NH^{bound}_{amide}	
GB-115***	1.59	0.91	1.23	1.76	0.57	65
(I)	2.75	1.35	–	under Arom	0.27	90

Notes * Δδ values represent the difference of chemical shifts δ in DMSO-d_6 and $CDCl_3$.
** β-turn fraction (%) in non-polar solvent was calculated as follows [14]: [β-turn] = 100–62Δδ for GB115 and [β-turn] = 100 –39Δδ for compound (I).
*** Due to the poor solubility of the GB-115 compound in pure $CDCl_3$, we used $CDCl_3$ containing 8% DMSO-d_6.

Table 3. Results of the conformational analysis of glycine- and proline-containing dipeptides with different *C*-terminal substitutes by ^{1}H NMR spectroscopy in solution

Compound		Activity	Value of Δδ *, ppm					Type of β-turn
Code	Formula		NH_{Trp}	NH_{Gly}	$NH_{\underline{NH}CH_3}$	NH^{free}_{amide} NH^{bound}_{amide}	NH^{ind}	
GB-115	$Ph(CH_2)_5CO$-**Gly**-Trp-$\mathbf{NH_2}$ **	+	0.91	1.23	–	1.76 **0.57**	1.59	β
(III)	$Ph(CH_2)_3CO$-**Gly**-Trp-$\mathbf{NHCH_3}$	–	**0.93**	1.54	1.30	–	2.48	γ
(IV)	$Ph(CH_2)_4CO$-**Gly**-Trp-$\mathbf{NHCH_3}$	–	**0.92**	1.52	1.29	–	2.67	γ
(I)	$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{NH_2}$	+	1.35	–	–	under Arom **0.27**	2.75	β
(II)	$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{OC_2H_5}$	–	**0.82**	–	–	–	2.77	γ
(V)	$Ph(CH_2)_5CO$-**Pro**-Trp-$\mathbf{NHCH_3}$	+	1.48	–	**0.80**	–	2.77	β

Notes: * Δδ values represent the difference of chemical shifts δ in DMSO-d_6 and $CDCl_3$.
** Due to the poor solubility of the GB-115 compound in pure $CDCl_3$, we used $CDCl_3$ containing 8% DMSO-d_6.

In biologically active peptides, β-turn and γ-turn structures occur most often [12, 13]. The β-turn structure (β-turn) is formed by four amino acid residues (*i*, *i* + 1, *i* + 2, *i* + 3) and is stabilized by IHB between the carbonyl group of the residue (*i*) (in our case, carbonyl of the phenylhexanoyl group) and $N^{\alpha}H$ proton of the residue (*i* + 3) (in our case, the *C*-terminal amide group). In the case of a γ-turn, the IHB is formed between the carbonyl group of the residue (*i*) and the $N^{\alpha}H$ proton of the residue (*i* + 2) (in our case, tryptophan). Thus, to determine the type of the turn structure in solution, it would be sufficient to reveal the involvement of a particular proton in the formation of IHB.

It is well known that the magnitude of the chemical shift of the proton involved in IHB changes insignificantly in the ^{1}H NMR spectrum on going from the polar solvent DMSO-d_6, which can participate in the formation of IHB, to nonpolar $CDCl_3$ [14]. It was shown (Table 2) that the difference in chemical shifts Δδ in GB-115 and (**I**) upon change of the solvents is small for one of the protons of the *C*-terminal amide group, which supposedly indicates the involvement of this proton in the formation of IHB. In the case of GB-115, this was also confirmed by the fact that the Δδ value for this proton did not depend on the concentration of the substance (data not shown). Because the Δδ values for NH_{Trp} for GB-115 and (**I**) were sufficiently great compared with those for other protons (Table 2), it was assumed that the formation of the γ-turn stabilized by IHB in these compounds is unlikely.

The calculation by the classical formula of Boussard and Marraud [14] showed that the share of the putative β-turn is 65% in the pool of GB-115 conformations and 90% in the case of the proline-containing GB-115 analogue (**I**) (Table 2). Thus, compound (**I**) exhibits a higher activity than GB-115 (Table 1) and simultaneously is more prone to form β-turns.

The data obtained suggest that the biologically active conformation of GB-115 and its proline-containing analogue (**I**) is the β-turn. These results are consistent with the fact that compounds having the γ-turn stabilized by the IHB with the involvement of $N^{\alpha}H$ proton of the tryptophan residue are inactive (Tables 1 and 3). According to the data of ^{1}H NMR spectroscopy in solution, among these are the methyl-

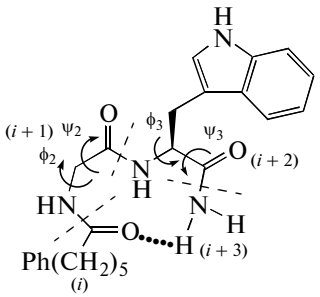


Fig. 1. β-Turn in the structure of GB-115.

amides of glycine-containing dipeptides (**III**) and (**IV**) and the ester of proline-containing dipeptide (**II**). At the same time, the proline-containing methylamide (**V**), which forms the β-turn stabilized by the IHB with the involvement of NH proton of the *C*-terminal *N*-methylamide group, is active.

Three β-conformations containing the IHB of the type $(i + 3) \rightarrow (i)$ are known: βI, βII, and βVI. The choice between these types of β-turns was made from the data of NOE ^1H-^1H double homonuclear resonance spectra of compounds GB-115 and (**I**). It is known that, for *N*-methylamides of *N*-acyl-substituted proline-containing dipeptides (R-Pro-Xaa-$NHCH_3$), which are structurally similar to our compounds [18, 19], the occurrence of the NOE between the $C^{\alpha}H$ proton of the residue $(i + 1)$ and $N^{\alpha}H$ proton of the residue $(i + 2)$ is evidence for the β-turn of type II. In the case of the proline-containing compound (**I**) in $CDCl_3$, the NOE (7%) was detected between $C^{\alpha}H_{Pro}$ and $N^{\alpha}H_{Trp}$ protons; in the case of GB-115, the NOE (4%) was detected between $C^{\alpha}H_{Gly}$ and $N^{\alpha}H_{Trp}$ protons. In addition, a characteristic feature of βII is the proximity of NH$(i + 2)$-NH$(i + 3)$, which is also confirmed by NOE: NH(Trp)-NH$(i + 3)$ is 3% in GB-115 and 5% in (**I**). Therefore, there is reason to believe that the β-turn of type II is also realized in these dipeptides in solution (Fig. 1).

The biologically active conformation of GB-115 was confirmed using the method of conformationally restricted analogues.

In analogue (**VI**), the γ-lactam fragment, which contributes to the formation of the type II β-turn [6, 7], was used as a spatial restraint. Compound (**VI**) was found to possess the anxiolytic activity, which compares well in effective doses with that of the starting dipeptide GB-115 (Table 1), indicating that the biologically active conformation of GB-115 is the type II β-turn.

On the other hand, the GB-115 *N*-methyl-substituted analogue, compound (**VII**) (Table 1), did not exhibit the anxiolytic activity. Because the *N*-methyl-substitution counteracts the formation of βII-turn in peptides [18–20], the lack of the activity in (**VII**) is also in favor of the βII-turn conformation of GB-115.

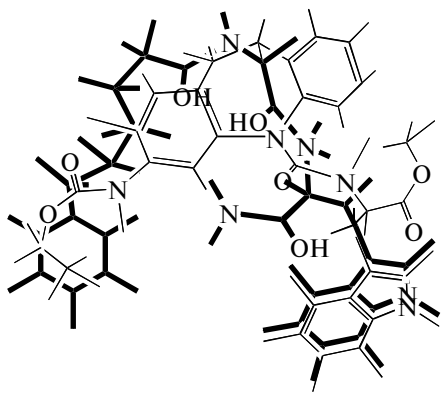


Fig. 2. Superposition of molecules of (**IX**) and GB-115 (solid lines) in Dreiding models.

Finally, we examined the spatially restricted compounds (**VIII**) and (**IX**), which have the dibenzoazepine structure. A pharmacological study showed that compound (**IX**) at doses comparable with those of GB-115 possesses the anxiolytic activity, whereas compound (**VIII**) is inactive (Table 1).

The two dibenzoazepine derivatives differ in that compound (**IX**) has a lateral substituent in the C3 position of the dibenzoazepine ring, which presumably participates in the interaction with the receptor and is required for the manifestation of the anxiolytic activity. According to the data of molecular simulation using the Dreiding models (Fig. 2), it is this fragment that imitates the hydrophobic phenyl group of GB-115 in the βII-turn conformation. The superposition of (**VIII**) and (**IX**) describes the three-dimensional structure of molecules in the unstrained state. In the model, the structure of the dipeptide GB-115 was fixed in the type II β-turn conformation.

To summarize, it can be concluded that the biologically active conformation of GB-115 is the type II β-turn.

EXPERIMENTAL

The peptide **GB-115** and its analogues **$Ph(CH_2)_5CO$-Pro-Trp-NH_2** (**I**), **$Ph(CH_2)_5CO$-Pro-Trp-OC_2H_5** (**II**), as well as the starting esters **$Ph(CH_2)_3CO$-Gly-Trp-OC_2H_5**, **$Ph(CH_2)_4CO$-Gly-Trp-OC_2H_5**, and **$Ph(CH_2)_5CO$-Gly-Trp-OC_2H_5** were obtained earlier [2, 5]. Commercial amino acids and their derivatives were from Reanal (Hungary and Acros Organics); reagents and solvents were from ZAO Ekos-1, Khimmed, Reakhim, Acros Organics, Fluka, and Lancaster. Amino acid esters were obtained by standard methods. *N*-acyl derivatives of amino acids were synthesized as described earlier [2]. *N*-Chlorocarbonyliminodibenzene and *N*-chlorocarbonyl-(3-ethoxycarbonylamino)iminodibenzene were kindly provided by VEB Arzneimittelwerk (Dresden). The solvents were purified and dried by standard methods.

^{1}H NMR spectra (δ, ppm, *J*, Hz) were recorded on a Bruker AC-250 spectrometer (Germany) in DMSO-d_6 and $CDCl_3$ solutions using tetramethylsilane as an internal standard. The melting point was determined in open capillaries and was not corrected. The specific optic rotation was measured on a Perkin-Elmer-241 polarimeter (England). Column chromatography was carried out on Kieselgel 100 (Merck, Germany). TLC was performed on Kieselgel 60 UV_{254} plates (Macherey-Nagel, Germany) using the following systems of solvents: chloroform–ethanol 9 : 1 (A); petroleum ether–ethyl acetate–ethanol 9 : 9 : 2 (B); dioxane–water 9 : 1 (C); butanol–acetic acid–water 5 : 1 : 2 (D); and isopropanol–ammonia 7 : 3 (E). Compounds were detected using iodine vapor and UV light. The elemental analysis was carried out on a device for carbon and hydrogen determination with four electric ovens (600–900°C, type MA-G/6P; LETO plant, Russia) in a flow of oxygen and on a device for nitrogen determination with three similar electric ovens in a flow of carbon dioxide. The data of the elemental analysis of compounds on the content of C, H, and N deviate from the theoretical data by no more than 0.4%.

Preparative HPLC was carried out using a Wellchrom 2001 chromatographic system (Knauer, Germany) on a Diasorb-130-C16T column (15 × 250 mm, C_{16}, 9 μm; ZAO BioKhimMak ST). The flow rate was 5 mL/min, and the detection was at 214 nm.

$Ph(CH_2)_3CO$-Gly-Trp-$NHCH_3$ (III). A solution of $Ph(CH_2)_3CO$-Gly-Trp-OC_2H_5 (0.41 g, 0.95 mmol) in methanol (10 mL) preliminarily saturated with gaseous methylamine at 0°C was allowed to stand at room temperature for 36 h in a closed container at atmospheric pressure (TLC control). The solvent was removed in vacuo, and the residue was triturated with dry ether. The precipitated crystals were filtered and dried over P_2O_5. Yield 0.28 g (70%); R_f 0.35 (A), mp 121–122°C, $[\alpha]_D^{20}$ −8.8° (*c* 0.1; ethanol); ^{1}H NMR (DMSO-d_6): 1.77 (2 H, m, $C^{\beta}H_2$ chain), 2.12 (2 H, t, *J* 7.1, $C^{\alpha}H_2$ chain), 2.54 (3 H, d, *J* 7.1, −NHC$\underline{H}_3$), 2.56 (2 H, t, *J* 7.0, $C^{\gamma}H_2$ chain), 2.92 and 3.11 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 3.58 and 3.75 (2 H, two dd, *J* 16.6, *J* 5.6, CH_2, Gly), 4.44 (1 H, m, $C^{\alpha}H$ Trp), 6.92–7.59 (10 H, m, ArH, CH indole), 7.88 (1 H, m, −N$\underline{H}$CH$_3$), 7.98 (1 H, d, *J* 8.3, NH Trp), 8.03 (1 H, t, *J* 5.7, NH Gly), 10.80 (1 H, s, NH indole); ^{1}H NMR ($CDCl_3$): 1.84 (2 H, m, $C^{\beta}H_2$ chain), 2.09 (2 H, t, *J* 7.2, $C^{\alpha}H_2$ chain), 2.56 (2 H, t, *J* 7.1, $C^{\gamma}H_2$ chain), 2.65 (3 H, d, *J* 7.0, −NHC$\underline{H}_3$), 3.13 and 3.30 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 3.76 and 3.82 (2 H, two dd, *J* 16.2, *J* 5.5, CH_2 Gly), 4.70 (1 H, m, $C^{\alpha}H$ Trp), 6.49 (1 H, t, *J* 5.8, NH Gly), 6.58 (1 H, br s, −N$\underline{H}$CH$_3$), 6.98 (1 H, s, CH indole), 7.05 (1 H, d, *J* 8.1, NH Trp), 7.0–7.61 (9 H, m, ArH), 8.32 (1 H, s, NH indole).

$Ph(CH_2)_4C(O)$-Gly-Trp-$NHCH_3$ (IV) was obtained similarly to compound (**III**) from $Ph(CH_2)_4C(O)$-Gly-Trp-OC_2H_5 (0.42 g, 0.93 mmol). Yield 0.25 g (62%); R_f 0.25 (A), mp 117–118°C, $[\alpha]_D^{20}$ −6.56° (*c* 0.1; ethanol); ^{1}H NMR (DMSO-d_6): 1.50 (4 H, m, $C^{\beta\gamma}H_2$ chain), 2.12 (2 H, t, *J* 7.1, $C^{\alpha}H_2$ chain), 2.54 (3 H, d, *J* 7.1, −NHC$\underline{H}_3$), 2.55 (2 H, t, *J* 6.9, $C^{\delta}H_2$ chain), 2.90 and 3.10 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 3.56 and 3.73 (2 H, two dd, *J* 16.5, *J* 5.6, CH_2 Gly), 4.42 (1 H, m, $C^{\alpha}H$ Trp), 6.92–7.35 (10 H, m, ArH, CH indole), 7.87 (1 H, q, −N$\underline{H}$CH$_3$), 7.97 (1 H, d, *J* 8.1, NH Trp), 8.01 (1 H, t, *J* 6.1, NH Gly), 10.81 (1 H, s, NH indole); ^{1}H NMR ($CDCl_3$): 1.84 (4 H, m, $C^{\beta\gamma}H_2$ chain), 2.09 (2 H, t, *J* 7.0, $C^{\alpha}H_2$ chain), 2.56 (3 H, d, *J* 7.1, −NHC$\underline{H}_3$), 2.65 (2 H, t, *J* 6.9, $C^{\delta}H_2$ chain), 2.76 and 3.82 (2 H, two dd, *J* 14.7, *J* 5.7, CH_2 Gly), 3.13 and 3.30 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 4.70 (1 H, m, $C^{\alpha}H$ Trp), 6.49 (1 H, br s, NH Gly), 6.58 (1 H, br s, −N$\underline{H}$CH$_3$), 6.90 (1 H, s, CH indole), 7.05 (1 H, d, *J* 8.1, NH, Trp), 7.00–7.61 (9 H, m, ArH), 8.32 (1 H, s, NH indole).

$Ph(CH_2)_5CO$-Pro-Trp-$NHCH_3$ (V) was obtained in the same way as compound (**III**) from compound (**II**) (0.50 g, 0.99 mmol) dissolved in methanol (10 mL) saturated with methylamine, and the reaction mixture was kept for 72 h (TLC control). Yield 0.35 g (72%), oil; R_f 0.53 (A), $[\alpha]_D^{20}$ −65° (*c* 0.4; methanol); ^{1}H NMR (DMSO-d_6) (ratio of *trans*/*cis* isomers 7/3): 1.18–2.20 (12 H, m, $C^{\alpha\beta\gamma\delta}H_2$ chains, *3*,*4*-CH_2 Pro), 2.44 (*cis*) and 2.55 (*trans*) (2 H, t, *J* 6.8, $C^{\varepsilon}H_2$ chain), 2.55 (3 H, d, *J* 6.8, −NHC$\underline{H}_3$), 2.94 (*cis*) and 3.05 (*cis*), 2.98 (*trans*) and 3.18 (*trans*) (2 H, two dd each, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 3.28–3.46 (2 H, m, *5*-CH_2 Pro), 4.18 (*trans*) and 4.29 (*cis*) (1 H, m, *2*-CH Pro), 4.40 (*trans*) and 4.55 (*cis*) (1 H, m each, $C^{\alpha}H$ Trp), 6.90–7.60 (10 H, m, ArH), 7.59 (*trans*) (1 H, q, −NHCH$_3$), 7.66 (*trans*) (1 H, d, *J* 8.2, NH Trp), 8.01 (*cis*) (1 H, m, −N$\underline{H}$CH$_3$), 8.27 (*cis*) (1 H, d, *J* 8.3, NH Trp), 10.81 (*cis*) and 10.83 (*trans*) (1 H, s, NH indole); ^{1}H NMR ($CDCl_3$): 1.07 (2 H, m, $C^{\gamma}H_2$ chains), 1.26 (2 H, m $C^{\beta}H_2$ chain), 1.58 (2 H, m, $C^{\delta}H_2$ chains), 1.88 (4 H, m, $C^{\alpha}H_2$ chain, *4*-CH_2 Pro), 2.09 (2 H, m, *3*-CH_2, Pro), 2.62 (2 H, t, $C^{\varepsilon}H_2$ chain), 2.73 (3 H, d, *J* 7.1, −NHC$\underline{H}_3$), 3.00 and 3.11 (2 H, two dd, *5*-CH_2, Pro), 3.12 and 3.56 (2 H, two dd, *J* 14.8, *J* 5.7, $C^{\beta}H_2$ Trp), 4.29 (1 H, dd, *2*-CH Pro), 4.75 (1 H, m, $C^{\alpha}H$ Trp), 6.18 (1 H, d, *J* 8.1, NH Trp), 6.79 (1 H, m, −N$\underline{H}$CH$_3$), 6.97 (1 H, s, CH indole), 7.07–7.59 (9 H, m, ArH), 8.06 (1 H, s, NH indole).

$Ph(CH_2)_5CO$-*D*-Met-Trp-OC_2H_5 (VIA) was obtained in the same way as compound (**VII**) (see below) from *N*-(6-phenylhexanoyl)-*D*-methionine (1.0 g, 3.1 mmol). Chloroform was removed in vacuo,

the resulting dark oil was kept at +5°C for 24 h and then triturated with diethyl ester, yielding a white crystalline precipitate. Crystals were carefully and quickly washed with ether and dried to obtain a white crystalline product. Yield 0.38 g (23%); R_f 0.71 (A), mp 104–106°C, $[\alpha]_D^{24}$ +12.24° (*c* 0.4; methanol); ^{1}H NMR ($CDCl_3$): 1.21 (3 H, t, *J* 7.0, $-OCH_2C\underline{H}_3$), 1.29 (2 H, m, $C^{\gamma}H_2$, chain), 1.57 (4 H, m, $C^{\beta\delta}H_2$ chain), 1.86 (2 H, m, $C^{\gamma}H_2$ Met), 2.07 (3 H, s, CH_3S Met), 2.09 (2 H, t, *J* 7.0, $C^{\alpha}H_2$ chain), 2.19 and 2.32 (2 H, two m, $C^{\beta}H_2$ Met), 2.55 (2 H, t, *J* 7.0, $C^{\varepsilon}H_2$ chain), 3.24 and 3.34 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 4.01 (2 H, q, *J* 7.2, $-OC\underline{H}_2CH_3$), 4.56 (1 H, m, $C^{\alpha}H$ Met), 4.81 (1 H, m, $C^{\alpha}H$ Trp), 6.37 (1 H, d, *J* 9.1, NH Met), 6.88 (1 H, d, *J* 8.1, NH Trp), 7.02 (1 H, s, CH indole), 7.05–7.56 (9 H, m, ArH), 8.30 (1 H, s, NH indole).

$[Ph(CH_2)_5CO$-*D*-Met(CH_3)-Trp-$OC_2H_5]\cdot I^-$ (VIB). A solution of $Ph(CH_2)_5CO$-*D*-Met-Trp-OC_2H_5 (**VIA**) (0.75 g, 1.4 mmol) in methyl iodide (7.5 mL) was stirred for 3 h at room temperature and left for 8 days. After the termination of the reaction (TLC control), the mixture was two-phasic; it consisted of a solution and a gel-like mass. The removal of excessive methyl iodide in vacuo followed by the drying over $CaCl_2$ and paraffin in vacuo (15 mmHg) yielded 0.825 g (87%) of product (**VIB**) as oil; R_f 0.1 (A), $[\alpha]_D^{24}$ +3.41° (*c* 0.4; methanol); ^{1}H NMR (DMSO-d_6): 1.09 (3 H, t, *J* 7.0, $-OCH_2C\underline{H}_3$), 1.24 (2 H, m, $C^{\gamma}H_2$ chain), 1.50 (4 H, m, $C^{\beta\delta}H_2$ chain), 1.8 (2 H, m, $C^{\gamma}H_2$ Met), 2.12 (2 H, t, *J* 6.9, $C^{\alpha}H_2$ chain), 2.53 (2 H, t, *J* 7.0, $C^{\varepsilon}H_2$ chain), 2.78 and 2.79 (6 H, two s, $(CH_3)_2S^+$), 3.03 and 3.07 (2 H, two m, $C^{\beta}H_2$ Met), 3.08 and 3.17 (2 H, two dd, *J* 14.9, *J* 5.7 , $C^{\beta}H_2$ Trp), 4.03 (2 H, q, *J* 7.1, $-OCH_2CH_3$), 4.45 (1 H, m, $C^{\alpha}H$ Met), 4.54 (1 H, m, $C^{\alpha}H$ Trp), 6.91–7.54 (10 H, m, ArH), 8.08 (1 H, d, *J* 9.0, NH Met), 8.49 (1 H, d, *J* 8.3, NH Trp), 10.31 (1 H, s, NH indole).

(2*S*)-2-{(3*R*)-3-[(6-Phenylhexanoyl)amino]-2-oxopyrrolidine-1-yl}-3-(1*H*-indole-3-yl)propionic acid ethyl ester (VI). Sodium hydride (0.087 g, 2.17 mmol) was added to a solution of $Ph(CH_2)_5CO$-*D*-Met$(CH_3)^+$-Trp-$OC_2H_5\cdot I^-$ (**VIB**) (0.735 g, 1.082 mmol) in 33.8 mL of the mixture DMF–CH_2Cl_2 1 : 1 cooled to 0°C and placed into a nitrogen flow. The mixture was stirred at 0°C for 2.5 h, after which ethyl acetate (7.3 mL) and then water (2.8 mL) were added. The mixture was left overnight at room temperature. The solvent was removed in vacuo, after which water (5.64 mL) and CH_2Cl_2 (5.64 mL) were added to the residue. The organic phase was separated, the solvent was removed in vacuo, and the residue was purified from contaminants by HPLC using a gradient of acetonitrile concentrations in 0.1% TFA (0–90%, 30 min) to give 0.19 g (0.41 mmol, 38%) of the derivative as oil. Then freshly distilled $SOCl_2$ (0.06 mL, 0.861 mmol) was added dropwise to the solution of oil in absolute ethanol (3 mL) at −10°C. The reaction mixture was stirred for 2 h at −10°C and kept for 48 h at room temperature, after which dry ether (10 mL) and activated charcoal (0.5 g) were added to the reaction mass under stirring. The reaction mass was then filtered, and the filtrate was evaporated in vacuo. The resulting ester in the form of yellow oil was purified from contaminants by HPLC using a gradient of acetonitrile concentrations in 0.1% TFA (0–90%, 30 min). Yield of the product as oil 0.100 g (50%, total yield 19%); R_f 0.81 (E), $[\alpha]_D^{24}$ +32.08° (*c* 4.8, methanol); ^{1}H NMR (DMSO-d_6): 1.05 (3 H, t, *J* 7.1, $-OCH_2C\underline{H}_3$), 1.23 (2 H, m, $C^{\gamma}H_2$ chain), 1.50 (4 H, m, $C^{\beta\delta}H_2$ chain), 1.62 (2 H, m, *4*-CH_2 lactam), 2.06 (2 H, t, *J* 7.1, $C^{\alpha}H_2$ chains), 2.53 (2 H, t, *J* 6.8, $C^{\varepsilon}H_2$ chain), 3.02 and 3.21 (2 H, two t, *J* 7.1, *5*-CH_2 lactam), 3.08 and 3.19 (2 H, two dd, *J* 14.9, *J* 5.7, $C^{\beta}H_2$ Trp), 4.10 (2 H, q, *J* 7.0, $-OC\underline{H}_2CH_3$), 4.23 (1 H, m, *3*-CH lactam), 4.70 (1 H, m, $C^{\alpha}H$ Trp), 6.92–7.35 (10 H, m, ArH), 8.12 (1 H, d, *J* 8.6, NH pyrrolidine), 10.81 (1 H, s, NH indole).

$Ph(CH_2)_5CO$-Gly-MeTrp-OC_2H_5 (VII). Isobutylchloroformate (0.13 mL, 1.0 mmol) and *N*-ethyl morpholine (0.12 mL, 1.0 mmol) were simultaneously added dropwise under stirring to a solution of *N*-(6-phenylhexanoyl)glycine (0.25 g, 1.0 mmol) in dry DMF (12 mL) cooled to −10°C. After stirring for 2–3 min at this temperature, a solution of N^{α}-methyltryptophan ethyl ester chlorohydrate (0.27 g, 1.0 mmol) cooled to −10°C and *N*-ethyl morpholine (0.13 mL, 1.0 mmol) in dry DMF (5.0 mL) was added. The reaction mixture was stirred for another 30 min at −10°C and for 1 h at room temperature, after which it was poured into cold water (200 mL). After 24 h at room temperature, oil formed, which was washed with water (2 × 30 mL) and dissolved in chloroform (30 mL). The solution was dried over anhydrous magnesium sulfate, the desiccant was filtered off, and the solvent was removed in vacuo. The resulting oil (yield 93%) was purified by HPLC using a gradient of acetonitrile concentrations (0–90%, 30 min). The yield after purification by HPLC was 0.24 g (50.5%) as oil; R_f 0.78 (A), $[\alpha]_D^{20}$ −23.3° (*c* 0.46; methanol); ^{1}H NMR (DMSO-d_6): 1.27 (3 H, t, *J* 7.1, $-OCH_2C\underline{H}_3$), 1.33 (2 H, m, $C^{\gamma}H_2$ chain), 1.62 (4 H, m, $C^{\beta\delta}H_2$ chain), 2.12 (*cis*) and 2.20 (*trans*) (2 H, m, $C^{\alpha}H_2$ chain), 2.57 (*cis*) and 2.59 (*trans*) (2 H, m, $C^{\varepsilon}H_2$ chain), 2.79 (*trans*) and 2.85 (*cis*) (3 H, s, $N^{\alpha}CH_3$), 3.25 and 3.47 (2 H, two dd, *J* 14.6, *J* 5.8, $C^{\beta}H_2$ Trp), 3.88 and 4.0 (2 H, two dd, *J* 16.6, *J* 5.6, CH_2 Gly), 4.21 (2 H, q, *J* 7.3, $-OC\underline{H}_2CH_3$), 4.56 (*cis*) and 5.30 (*trans*) (1 H, dd each, *J* 14.9, *J* 8.1 and *J* 14.9, *J* 7.6, respectively, $C^{\alpha}H$ Trp), 6.37 (*cis*) and 6.48 (*trans*) (1 H, t, *J* 5.6 and *J* 5.7, respectively, NH Gly), 6.80 (1 H, d, *J* 8.3, NH Trp),

6.95–7.60 (10 H, m, ArH), 8.11 (*trans*) and 8.16 (*cis*) (1 H, s, NH indole).

(2*S*)-2-[(10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-ylcarbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester (VIII). A solution of tryptophan methyl ether hydrochloride (0.43 g, 1.6 mmol) and *N*-ethyl morpholine (0.28 mL, 2.2 mmol) in DMF (5 mL) was added under stirring to a solution of 10,11-dihydrodibenzo[*b, f*]azepin-5-carbonylchloride (**VIIIA**) (0.39 g, 1.5 mmol) in DMF (5 mL) cooled to 0°C. The reaction mass was stirred for 30 min at room temperature and for 3 h upon heating to 40°C, poured into a container with distilled water (150 mL), and cooled to 0°C. The precipitate was filtered, air-dried, and recrystallized from toluene. Yield 0.20 g (30.3%); R_f 0.9 (A), mp 157–158°C, $[\alpha]_D^{22}$ –5.43° (*c* 0.4; methanol); ^{1}H NMR (DMSO-d_6): 2.92 (4 H, br s, $-CH_2CH_2-$), 3.12 (2 H, d, *J* 5.7, $C^\beta H_2$ Trp), 3.60 (3H, s, $-OCH_3$), 4.47 (1 H, m, $C^\alpha H$ Trp), 5.43 (1 H, d, *J* 8.1, NH Trp), 6.84–7.42 (13 H, m, ArH), 10.89 (1 H, s, NH indole).

(2*S*)-2-[({3-[(ethoxycarbonyl)amino]-10,11-dihydro-5*H*-dibenzo[*b, f*]azepin-5-yl}carbonyl)amino]-3-(1*H*-indole-3-yl)propionic acid methyl ester (IX). A solution of tryptophan methyl ether hydrochloride (0.43 g, 1.6 mmol) and *N*-ethyl morpholine (0.28 mL, 2.2 mmol) in DMF (7 mL) was added under stirring to a solution of 10,11-dihydrobenzo[*b, f*]azepino-[3-ethoxycarbonyl)amino]-5-carbonylchloride (**IXA**) (0.52 g, 1.5 mmol) in DMF (5 mL). The reaction mass was stirred for 30 min at room temperature and for 3 h upon heating to 45–50°C after which it was allowed to stand for 15 h at room temperature. Then the reaction mass was poured into a container with distilled water (150 mL), and cooled to 0°C. The precipitate was filtered off, air-dried, and recrystallized from ethyl acetate. Yield 0.4 g (50.7%); R_f 0.75 (A), mp 155–157°C, $[\alpha]_D^{22}$ +7.03° (*c* 0.4; methanol); ^{1}H NMR (DMSO-d_6): 1.22 (3 H, t, *J* 7.0, $-OCH_2C\underline{H}_3$), 2.85 (4 H, m, $-CH_2CH_2-$), 3.11 (2 H, d, *J* 5.7, $C^\beta H_2$ Trp), 3.60 (3 H, s, $-OCH_3$), 4.10 (2 H, q, *J* 7.2, $-OC\underline{H}_2CH_3$), 4.47 (1 H, m, $C^\alpha H$ Trp), 5.44 (1 H, d, *J* 8.1, NH Trp), 6.85–7.47 (12 H, m, ArH), 9.61 (1 H, s, NH ethoxycarbamoyl), 10.85 (1 H, s, NH indole).

^{1}H NMR study. ^{1}H NMR spectra were recorded at room temperature on a Bruker AC-250 spectrometer (Germany) in $CDCl_3$ and DMSO-d_6 solutions, using tetramethylsilane as an internal standard. Solutions with a concentration of 10–20 mg/mL were examined. Signals were assigned on the basis of literature data on ^{1}H NMR spectra of the corresponding amino acids [21] and the method of homonuclear double-resonance, which enables the detection of groups of spin–spin-coupled protons. To determine IHBs, the dependence of the chemical shifts of protons on the concentration of a substance in $CDCl_3$ and the nature of solvent was studied using $CDCl_3$/DMSO-d_6 mixtures. Spin–spin coupling constants ^{3}J in the system NH-C^αH were measured from the signal of NH protons. The magnitude of NOE in one-dimensional NMR spectra was determined by selective low-intensity irradiation at the frequency of the resonance chosen. The quantity $NOE_j(i)$ was defined as the ratio of the change in the integral intensity of signal j in the NMR spectrum upon the saturation of nucleus I (I_j) to the integral intensity of signal *j* in the absence of saturation (I_j^0): $NOE_j(i) = (I_j - I_j^0)/I_j^0$.

The biological activity of peptides in vivo was studied on white mongrel male rats weighing 200–270 g. Standard granulated feed and water were given ad libitum. Ethical rules for the animal care formulated in the directions of the Council of European Community 86/609/EEC were followed. Rats were housed in a vivarium in standard plastic cages (60 × 40 × 20), eight individuals in each. Each experimental rat was subjected for three days preceding the experiment to a "handling" procedure. Animals were placed in an experimental room three to four hours prior to the beginning of the experiment, which was carried out between 16 and 20 h of the local time. Experiments were performed in a darkened room; the light source (60-watt bulb) was screened so that the device was illuminated only with diffuse reflected light. Water–Tween solutions of compounds were prepared ex tempore and injected to rats intraperitoneally 15 min prior to the testing in the maze. Control animals received an equal volume of distilled water.

The EPM test in the basic modification [8] was used as an experimental model. The maze had the following characteristics: the length of each of four maze arms 50 cm, the width 15 cm, the height of the light-proof skirtings of two opposite closed arms 15 cm; the dimensions of the central stage 15 × 15 cm; the maze was elevated over the floor at a height of 75 cm. At the beginning of the experiment, rats were placed in the center of the maze and oriented randomly relative to the entrance to the arm. The time of assessing the behavior of animals was 5 min. The following parameters were recorded: the number of runs in all arms, the number of runs into open arms, the time of stay in all arms, and the time of stay in open arms.

The statistical processing of results was performed using the nonparametric Mann–Whitney U-test (software package Complete statistical system, version B640, Statsoft, United States). The data are presented as the means +/– error of the means. The results were considered as significant at $p < 0.05$.

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