MOLECULAR DESIGN, SYNTHESIS AND NEUROLEPTIC ACTIVITY OF DIPEPTIDE ANALOGS OF SULPIRIDE

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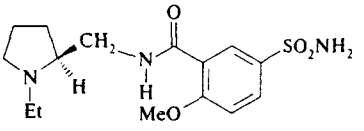
Some nonpeptide psychotropic drugs are known to represent the ligands of the corresponding neuropeptide receptors. For example, morphine acts via the enkephalin receptors [1], while the benzodiazepine tranquilizers are the ligands of the DBI peptide receptors [2]. This circumstance gives us a possibility to synthesize biologically active short-chain peptides that are structural analogs of the known neurotropic agents and may serve as base structures for the development of new groups of psychotropic drugs.

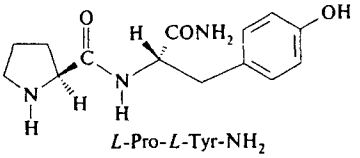
Previously we have used this approach to obtain two promising groups of peptide nootropic drugs, pyroglutamyl- and N-acylprolyl-containing dipeptides, based on the classical pyrrolidone nootropic drug pyracetam [3 – 5].

Below we present data on the molecular design, synthesis, and the study of neuroleptic activity of dipeptide analogs of the drug sulpiride belonging to the series of atypical neuroleptics. According to clinical experience, this drug is characterized as producing a "regulatory" effect on the central nervous system and having a low ability to induce extrapyramidal diseases in the patients [6, 7].

Sulpiride, or 5-sulfamoyl-N-[(1-ethyl-2-pyrrolidinyl)-methyl]-2-methoxybenzamide, contains structural elements known to be present in natural peptides: a 5-membered pyrrolidine cycle with positively charged (under physiological conditions) nitrogen atom (similar to that in the side radical of the N-ended proline) and a substituted benzene ring capable of imitating the hydroxyphenyl group of tyrosine. The amide group of sulpiride may play the role of a peptide link. Therefore, the Pro–Tyr–NH_2 dipeptide may be considered one of the simplest peptide analogs of sulpiride.



(*S*)-Sulpiride



L-Pro-*L*-Tyr-NH_2

A peptidergic mechanism of the sulpiride activity is confirmed, besides the above structural considerations, by a U-shaped curve of the dose – effect relationship (see Table 1), which is characteristic of most neuroleptics and reflects their neuromodulator properties.

Superposition of sulpiride and Pro–Tyr–NH_2 molecules (within the Draiding model) showed good coincidence of the aryl nuclei and pyrrolidine cycles (including nitrogen atoms) and a satisfactory agreement between the positions of nitrogen in the amide and peptide groups for unstressed conformations. This conclusion was corroborated by computer simulation of the conformations of sulpiride and Pro-Tyr-NH_2 molecules performed by the method of molecular dynamics using the MMX force field and the PC MODEL program (these results will be reported in a special publication).

For the experimental verification of our hypothesis, we have synthesized several Pro-Tyr-NH_2 diastereomers and analogs. The Pro-Tyr-NH_2 diastereomers were obtained in the form of trifluoroacetate salts by acidolysis of the corresponding BOC-derivatives of dipeptides. The latter were prepared using the method of mixed anhydrides under the reaction conditions described by Andersen [8]:

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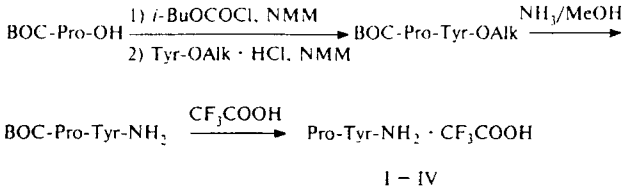
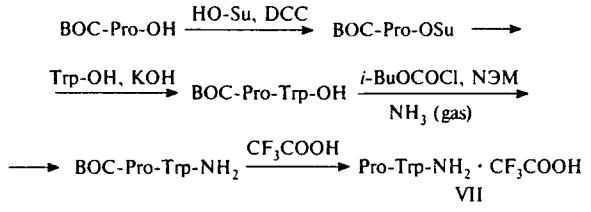


BOC — *tert*-butyloxycarbonyl;
NMM — N-methylmorpholine, Alk-Me, or Et.

A similar procedure was used to obtain dipeptides of the types Gly-*L*-Tyr-NH_2 (V) and *L*-Pro-*L*-Phe-NH_2 (VI). The tryptophan analog *L*-Pro-*L*-Trp-NH_2 was synthesized by the method of activated esters (using N-hydroxysuccinimide derivative) followed by acidolysis of the BOC-dipeptide amide:



HO-Su — N-hydroxysuccinimide;
DCC — dicyclohexylcarbodiimide;
NEM — N-ethylmorpholine.

The neuroleptic activity of the synthesized peptides was assessed by the degree of inhibition of the apomorphine-induced verticalization in mice [9] and the recovery of extrapolated avoidance behavior in an extremal situation against the madopar (*L*-DOPA + benzerazide) induced disturbance in rats [10]. The latter test has a sensitivity comparable to that of the former, while markedly exceeding it with respect to selectivity [11].

Intraperitoneal injections of Pro-Tyr-NH_2 dipeptide (I) in the dose range 3 – 16 mg/kg reduced the apomorphine-induced verticalization, the effect exceeding that of sulpiride (Table 1). Similar results were obtained in the extrapolated avoidance test.

Study of the effect of the amino acid configuration on the activity of Pro-Tyr-NH_2 dipeptides showed that substituting *D*-Tyr for *L*-Tyr (compounds II, IV) leads to the complete loss of activity on both tests. At the same time, the change from *L*-Pro to *D*-Pro (compounds III) has no significant effect on the activity. Thus, it is only the configuration of tyrosine residue that affects the biological activity manifestations of Pro-Tyr-NH_2. The stereoselectivity of the neuroleptic effect of dipeptide I is evidence of the receptor mechanism of its action.

We have also studied the role of the nature of amino acid in dipeptide I. Replacement of the proline residue for glycine (a common fragment of the natural amino acids free of side radicals) leads to the loss of activity (compound V). This result indicates that the side radical of proline participates in the proposed interaction with the receptor. Replacement of the tyrosine residue by phenylalanine (i.e., by a residue having similar structure) in compound VI retains the neuroleptic activity, whereas the substitution of tryptophan (a different aromatic amino acid residue) in compound VII results in the loss of activity. These data are indicative of a restricted size of the receptor site for the side radical of the second amino acid.

EXPERIMENTAL CHEMICAL PART

The melting temperatures were determined using open capillaries (without correction). The ^1H NMR spectra were obtained on a Bruker AC-250 spectrometer (Germany) using TMS as the internal standard. The specific optical rotation

TABLE 1. Neuroleptic Activity of Sulpiride Dipeptide Analogs I – VII*

Compound	Dose, mg/kg (i.p.)	Apomorphine-induced verticalization in mice		Recovery of madopar-violated extrapolated avoidance reflex in rats	
		vertica-lization, %	activity, %	dive-under, %	activity, %
Intact (NaCl physiol. solution)	–	0	100	80	100
Control (apomorphine 5 mg/kg, s.c.)	–	100	0	–	–
Control (100 mg/kg *L*-DOPA + 25 mg/kg benzerazide, s.c.)	–	–	–	20	0
L-Pro-*L*-Tyr-NH_2 (I)	1.0	99	40	33	
	2.0	85	15	64**	73**
	3.0	52**	48**	...	...
	4.0	37**	63**	67**	78**
	8.0	52**	48**	...	...
	16.0	66**	34**	...	...
L-Pro-*D*-Tyr-NH_2 (II)	2.0	...	...	20	0
	3.0	...	...	40	33
	4.0	98	2	...	...
	8.0	97	3	...	...
D-Pro-*L*-Tyr-NH_2 (III)	4.0	33*	67**	50**	50**
	6.0	58**	42**	...	...
	8.0	49**	51**	60**	67**
D-Pro-*D*-Tyr-NH_2 (IV)	1.0	...	...	20	0
	2.0	92	8	...	...
	3.0	...	...	20	0
	4.0	103	–3	...	...
Gly-*L*-Tyr-NH_2 (V)	8.0	81	19	50	50
L-Pro-*L*-Phe-NH_2 (VI)	8.0	65**	35**	78**	97**
L-Pro-*L*-Trp-NH_2 (VII)	4.0	85	15	...	...
Sulpiride	4.0	...	...	20	0
	8.0	79	21	60**	67**
	16.0	63**	37**	75**	91**
	32.0	70**	30**	40	33
	64.0	69**	31**	...	...

* In the form of trifluoroacetate.
** $p < 0.05$ against the control.

was measured with a Perkin-Elmer Model 241 polarimeter (England). The TLC chromatograms were obtained using Kieselgel 60F-254 (Merck, Germany), Silufol UV-254 (Serva, Germany), Silufol Kavalier (Czech Republic), and Silica Gel (Eastman Kodak, USA) plates; the spots were visualized by exposure to iodine vapors. The column chromatography was performed on a Kieselgel 100 (Merck) column. The solvents were purified and dehydrated by conventional methods. The data of elemental analyses coincided with the results of analytical calculations according to the empirical formulas.

The esters of amino acids were obtained in the form of hydrochlorides as described in [12].

***L*-Tyrosine methyl ester hydrochloride (*L*-Tyr-OMe·HCl).** Yield 75%; m.p., 179 – 180°C; $[\alpha]_D^{20}$, + 72° (*c* 3; pyridine); R_f, 0.69 (Silufol Serva; isopropyl alcohol – 25% ammonia, 7 : 3); $C_{10}H_{14}ClNO_3$. Published data: yield, 92%; m.p., 190°C; $[\alpha]_D^{22.5}$, + 74.3° (*c* 3; pyridine); R_f, 0.95 (methyl ethyl ketone – pyridine – water, 60 : 15 : 25) [13].

***L*-Tyrosine ethyl ester hydrochloride (*L*-Tyr-OEt·HCl).** Yield 86%; m.p., 164 – 165°C; $[\alpha]_D^{20}$, – 6.8° (*c* 2; water); $C_{11}H_{16}ClNO_3$. Published data: m.p., 166°C; [13].

***D*-Tyrosine methyl ester hydrochloride (*D*-Tyr-OMe·HCl).** Yield 70%; m.p., 183 – 185°C; $[\alpha]_D^{20}$, – 72° (*c* 3; pyridine); R_f, 0.69 (Silufol Serva; isopropyl alcohol – 25% ammonia, 7 : 3); $C_{10}H_{14}ClNO_3$.

***D*-Tyrosine ethyl ester hydrochloride (*D*-Tyr-OEt·HCl).** Yield 63%; m.p., 164 – 165°C; $[\alpha]_D^{20}$, + 5.5° (*c* 2; water); R_f, 0.75 (Silufol Kavalier; isopropyl alcohol – 25% ammonia, 7 : 3); $C_{11}H_{16}ClNO_3$.

***L*-Phenylalanine ethyl ester hydrochloride (*L*-Phe-OEt·HCl).** Yield 77%; m.p., 149 – 150°C; $[\alpha]_D^{20}$, – 8° (*c* 3; water); R_f, 0.70(Silufol Kavalier; isopropyl alcohol – 25% ammonia, 7 : 3); $C_{11}H_{16}ClNO_2$. Published data: m.p., 154 – 155°C; $[\alpha]_D^{20}$, – 7.6° (*c* 3; water) [14].

N-*tert*-Butyloxycarbonylamino acids were synthesized by procedures similar to those described in [15].

N-*tert*-Butyloxycarbonyl-*L*-proline (BOC-*L*-Pro-OH). Yield, 78%; m.p., 130 – 131°C; $[\alpha]_D^{20}$, – 59° (*c* 2; acetic acid); R_f, 0.77 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); $C_{10}H_{17}NO_4$. Published data: m.p., 136 – 137°C; $[\alpha]_D^{25}$, – 60.2° (*c* 2; acetic acid) [16].

N-*tert*-Butyloxycarbonyl-*D*-proline (BOC-*D*-Pro-OH). Yield, 77%; m.p., 131 – 132°C; $[\alpha]_D^{20}$, + 61° (*c* 2; acetic acid); R_f, 0.71 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); $C_{10}H_{17}NO_4$.

N-*tert*-Butyloxycarbonylglycine (BOC-Gly-OH). Yield, 81%; m.p., 82 – 84°C; R_f, 0.21 (Silufol Kavalier, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.38 (*c*, 9H, $(CH_3)_3C$), 3.60 (m, 2H, $NHCH_2$), 7.06 (m, 1H, $NHCH_2$), 12.40 (s, 1H, OH); $C_7H_{13}NO_4$. Published data: m.p., 88.5 – 89°C; [17].

General method for the synthesis of N-*tert*-butyloxycarbonyl dipeptide esters.

To a solution of 10 mmole of a carboxy component (BOC-amino acid) in 50 ml of chloroform (or ethyl acetate) is added with stirring and cooling (– 15°C) dropwise (from different dropping funnels) 10 mmole of N-ethylmorpholine and 10 mmole of isobutylchloroformate. After 2 – 3 min, to this mixture is slowly added 10 mmole of the corresponding amino component (amino acid ester hydrochloride) in 10 ml DMF with 10 mmole of N-ethylmorpholine. The reaction mixture is kept for 30 min at – 15°C and then for 1 h at room temperature. The precipitate is dissolved in chloroform, washed successively with 5% $NaHCO_3$, water, 1 N HCl, and water to pH ~ 3, and dried over $MgSO_4$. Finally, the solvent is distilled off and the residue is chromatographically purified on a column eluted with chloroform.

***tert*-Butyloxycarbonyl-*L*-prolyl-*L*-tyrosine methyl ester (BOC-*L*-Pro-*L*-Tyr-OMe)** was obtained from BOC-*L*-Pro-OH and *L*-Tyr-OMe · HCl with a yield of 82%. The product appears as a white crystalline substance; m.p., 106 – 110°C; $[\alpha]_D^{20}$, – 49.5° (*c* 0.35; chloroform); R_f, 0.63 (Kieselgel, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.24, 1.37 (2s, 9H, $(CH_3)_3C$), 1.60 – 2.12 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.75 – 3.98 (m, 2H, $C^\beta H_2$ Tyr), 3.15 – 3.50 (m, 2H, $C^\delta H_2$Pro), 3.56, 3.60 (2s, 3H, OCH_3), 4.05, 4.15 (2dd, 1H, C^αHPRo), 4.41 (m, 1H, C^αH Tyr), 6.65, 7.00 (2m, 4H, C_6H_4 Tyr), 8.11, 8.16 (2d, 1H, NH Tyr), 9.18 (s, 1H, OH Tyr); $C_{20}H_{28}N_2O_6$. Published data: $[\alpha]_D^{25}$, – 37.6° (*c* 1; ethanol) [18].

***tert*-Butyloxycarbonyl-*L*-prolyl-*D*-tyrosine methyl ester (BOC-*L*-Pro-*D*-Tyr-OMe)** was obtained from BOC-*L*-Pro-OH and *D*-Tyr-OMe · HCl with a yield of 87%. The product has the form of an oil; R_f, 0.60 (Kieselgel, chloroform – ethanol, 9 : 1); $[\alpha]_D^{20}$, – 72° (*c* 0.23; chloroform); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.29, 1.39 (2s, 9H, $(CH_3)_3C$), 1.41 – 2.09 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.67 – 2.99 (m, 2H, $C^\beta H_2$ Tyr), 3.11 – 3.35 (m, 2H, $C^\delta H_2$Pro), 3.59, 3.62 (2s, 3H, OCH_3), 4.05, 4.09 (2dd, 1H, C^αH Pro), 4.46 (m, 1H, C^αH Tyr), 6.64, 7.00 (2m, 4H, C_6H_4 Tyr), 8.09, 8.26 (2d, 1H, NH Tyr), 9.24 (s, 1H, OH Tyr); $C_{20}H_{28}N_2O_6$.

***tert*-Butyloxycarbonyl-*D*-prolyl-*L*-tyrosine methyl ester (BOC-*D*-Pro-*L*-Tyr-OMe)** was obtained from BOC-*D*-Pro-OH and *L*-Tyr-OMe · HCl with a yield of 86%. The product has the form of an oil; $[\alpha]_D^{20}$, + 72° (*c* 0.23; chloroform); R_f, 0.61 (Kieselgel, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.29, 1.39 (2s, 9H, $(CH_3)_3C$), 1.41 – 2.09 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.67 – 2.99 (m, 2H, $C^\beta H_2$ Tyr), 3.11 – 3.35 (m, 2H, $C^\delta H_2$Pro), 3.59, 3.62 (2s, 3H, OCH_3), 4.05, 4.09 (2dd, 1H, C^αHPro), 4.46 (m, 1H, C^αH Tyr), 6.64, 6.97 (2m, 4H, C_6H_4 Tyr), 8.09, 8.26 (2d, 1H, NH Tyr), 9.22 (s, 1H, OH Tyr); $C_{20}H_{28}N_2O_6$.

***tert*-Butyloxycarbonyl-*D*-prolyl-*D*-tyrosine ethyl ester (BOC-*D*-Pro-*D*-Tyr-OEt)** was obtained from BOC-*D*-Pro-

OH and *D*-Tyr-OEt · HCl with a yield of 90%. The product has the form of an oil; R_f, 0.75 (Kieselgel, chloroform – ethanol, 9 : 1); $[\alpha]_D^{20}$, + 45.3° (*c* 0.39; chloroform); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.08, 1.11 (2t, 3H, CH_3CH_2O), 1.21, 1.37 (2s, 9H, $(CH_3)_3C$), 1.15 – 1.59 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.76 – 3.00 (m, 2H, $C^\beta H_2$ Tyr), 3.15 – 3.35 (m, 2H, $C^\delta H_2$Pro), 4.00, 4.03 (2qq, 2H, CH_3CH_2O), 4.11 (m, 1H, C^αHPro), 4.31, 4.36 (2m, 1H, C^αH Tyr), 6.63, 7.00 (2m, 4H, C_6H_4 Tyr), 8.10, 8.15 (2d, 1H, NH Tyr), 9.23, 9.25 (2s, 1H, OH Tyr); $C_{21}H_{30}N_2O_6$.

***tert*-Butyloxycarbonyl-*L*-prolyl-*L*-phenylalanine ethyl ester (BOC-*L*-Pro-*L*-Phe-OEt)** was obtained from BOC-*L*-Pro-OH and *L*-Phe-OEt · HCl with a yield of 79%. The product has the form of an oil; R_f, 0.63 (Silufol Kavalier, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 + CF_3COOD (δ, ppm): 1.11, 1.15 (2t, 3H, CH_3CH_2O), 1.22, 1.39 (2s, 9H, $(CH_3)_3C$), 1.68 (m, 2H, $C^\gamma H_2$Pro), 1.73 – 2.03 (m, 2H, $C^\beta H_2$Pro), 2.97, 3.06 (2dd, 2H, $C^\beta H_2$Phe), 3.25 – 3.35 (m, 2H, $C^\delta H_2$Pro), 4.06 (q, 2H, CH_3CH_2O), 4.13 (m, 1H, C^αHPRo), 4.50 (m, 1H, C^αHPhe), 7.25 (m, 5H, C_6H_5Phe), 8.22, 8.27 (2d, 1H, NHr).

N-*tert*-Butyloxycarbonylglycyl-*L*-tyrosine ethyl ester (BOC-Gly-*L*-Tyr-OEt) was obtained from BOC-Gly-OH and *L*-Tyr-OEt · HCl with a yield of 66%. The product has the form of an oil; R_f, 0.62 (Silufol Kavalier, chloroform – ethanol, 8 : 2); R_f, 0.4 (Silufol kavalier, ethyl acetate – petroleum ether – ethanol, 5 : 5 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.12 (t, 3H, CH_3CH_2O), 1.37 (2s, 9H, $(CH_3)_3C$), 2.83 (m, 2H, $C^\beta H_2$ Tyr), 3.52 (m, 2H, $C^\alpha H_2$Gly), 4.02 (q, 2H, CH_3CH_2O), 4.36 (m, 1H, C^αH Tyr), 6.65, 6.96 (2m, 4H, C_6H_4 Tyr), 6.92 (t, 1H, NHGly), 8.09 (d, 1H, J 7.3 Hz, NH Tyr), 9.25 (s, 1H, OH Tyr); $C_{18}H_{26}N_2O_6$.

General method for the synthesis of N-*tert*-butyloxycarbonyl dipeptide amides.

A solution of 5 mmole of a BOC-substituted dipeptide in 50 ml of absolute alcohol (methanol or ethanol for ethyl and methyl esters, respectively) is saturated with ammonia at 0°C and allowed to stand for 2 days at room temperature. Then the solvent is distilled off in vacuum and the product is purified on a chromatographic column (eluted first with chloroform and then with a chloroform – ethanol (8 : 2) mixture.

***tert*-Butyloxycarbonyl-*L*-prolyl-*L*-tyrosine amide (BOC-*L*-Pro-*L*-Tyr-NH_2)** was obtained from BOC-*L*-Pro-*L*-Tyr-OMe with a yield of 60%; m.p., 72 – 75°C; R_f, 0.33 (Kieselgel, chloroform – ethanol, 9 : 1); $[\alpha]_D^{20}$, – 49.7° (*c* 0.38; chloroform); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.22, 1.39 (2s, 9H, $(CH_3)_3C$), 1.56 – 2.10 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.60 – 3.00 (m, 2H, $C^\beta H_2$ Tyr), 3.20 – 3.40 (m, 2H, $C^\delta H_2$Pro), 4.01 (dd, 1H, J 8.8 Hz, J 3.5 Hz, C^αHPro), 4.30, 4.41 (2m, 1H, C^αH Tyr), 6.62, 6.98 and 6.62, 7.00 (4m, 4H, C_6H_4 Tyr), 7.00, 7.08 (2s, 2H, NH_2), 7.70, 7.75 (2d, 1H, NH), 9.18, 9.20 (2s, 1H, OH); $C_{19}H_{27}N_3O_5$.

***tert*-Butyloxycarbonyl-*L*-prolyl-*D*-tyrosine amide (BOC-*L*-Pro-*D*-Tyr-NH_2)** was obtained from BOC-*L*-Pro-*D*-Tyr-OMe with a yield of 46%; m.p., 79 – 80°C; R_f, 0.36 (Kieselgel, chloroform – ethanol, 9 : 1); $[\alpha]_D^{20}$, – 34° (*c* 0.3; chloroform); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.28, 1.38 (2s, 9H, $(CH_3)_3C$), 1.55 – 2.08 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.64, 2.84 and 2.64, 2.98 (2dd, 2H, $C^\beta H_2$ Tyr), 3.10 – 3.32 (m, 2H, $C^\delta H_2$Pro), 4.05 (dd, 1H, C^αHPro), 4.26, 4.46 (2m, 1H, C^αH Tyr), 6.62, 6.98 (2m, 4H, C_6H_4 Tyr), 7.05, 7.37 and 7.16, 7.28 (4s, 2H, NH_2), 7.88, 8.12 (2d, 7H, J 8.9 Hz, J 8.6 Hz, NH Tyr), 9.15 (s, 1H, OH Tyr); $C_{19}H_{27}N_3O_5$.

***tert*-Butyloxycarbonyl-*D*-prolyl-*L*-tyrosine amide (BOC-*D*-Pro-*L*-Tyr-NH_2)** was obtained from BOC-*D*-Pro-*L*-Tyr-OMe with a yield of 40%; m.p., 76 – 78°C; $[\alpha]_D^{20}$, + 33.2° (*c* 0.3; chloroform); R_f, 0.36 (Kieselgel, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.28, 1.38 (2s, 9H, $(CH_3)_3C$), 1.55 – 2.08 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.64, 2.84 and 2.64, 2.98 (4dd, 2H, $C^\beta H_2$ Tyr), 3.10 – 3.32 (m, 2H, $C^\delta H_2$Pro), 4.05 (dd, 1H, C^αHPro), 4.26, 4.46 (2m, 1H, C^αH Tyr), 6.62, 6.98 (2m, 4H, C_6H_4 Tyr), 7.05, 7.37 and 7.16, 7.28 (4s, 2H, NH_2), 7.88, 8.12 (2d, 7H, J 8.9 Hz, J 8.6 Hz, NH Tyr), 9.15 (s, 1H, OH Tyr); $C_{19}H_{27}N_3O_5$.

***tert*-Butyloxycarbonyl-*D*-prolyl-*D*-tyrosine amide (BOC-*D*-Pro-*D*-Tyr-NH_2)** was obtained from BOC-*D*-Pro-*D*-Tyr-OEt with a yield of 77%; m.p., 72 – 75°C; $[\alpha]_D^{20}$, +47.9° (*c* 0.4; ethanol); R_f, 0.33 (Kieselgel, chloroform – ethanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.22, 1.39 (2s, 9H, $(CH_3)_3C$), 1.56 – 2.10 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.60 – 3.00 (m, 2H, $C^\beta H_2$ Tyr), 3.20 – 3.40 (m, 2H, $C^\delta H_2$ Pro), 4.00 (dd, 1H, C^αH PRo), 4.30, 4.41 (2m, 1H, C^αH Tyr), 6.62, 7.00 (2m, 4H, C_6H_4 Tyr), 7.00, 7.08 and 7.16, 7.30 (4s, 2H, NH_2), 7.70, 7.75 (2d, 1H, NH Tyr), 9.18, 9.20 (2s, 1H, OH Tyr); $C_{19}H_{27}N_3O_5$.

***tert*-Butyloxycarbonyl-*L*-prolyl-*L*-phenylalanine ethyl ester (BOC-*L*-Pro-*L*-Phe-NH_2)** was obtained from BOC-*L*-Pro-*L*-Phe-OEt with a yield of 81% (upon trituration with petroleum ether); m.p., 132 – 135°C; R_f, 0.54 (Silufol Kavalier, chloroform – ethanol, 9 : 1); $[\alpha]_D^{20}$, – 69.5° (*c* 0.4; methanol); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.2, 1.4 (2s, 9H, $(CH_3)_3C$), 1.62, 1.99 (2m, 2H, $C^\beta H_2$ Pro), 1.62 (m, 2H, $C^\gamma H_2$ Pro), 2.7 – 3.1 (m, 2H, $C^\beta H_2$ Phe), 3.1 – 3.6 (m, 2H, $C^\delta H_2$ Pro), 4.0 (m, 1H, C^αH Pro), 4.46 (m, 1H, C^αH Phe), 7.0 – 7.5 (m, 5H, C_6H_5 Phe), 7.84 (d, 1H, NH Phe); $C_{19}H_{27}N_3O_4$.

N-*tert*-Butyloxycarbonylglycyl-*L*-tyrosine amide (BOC-Gly-*L*-Tyr-NH_2) was obtained from BOC-Gly-*L*-Tyr-OEt with a yield of 76%; m.p., 135 – 138°C; R_f, 0.3 (Silufol Kavalier, chloroform – ethanol, 8 : 2); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.36 (s, 9H, $(CH_3)_3C$), 2.68, 2.87 (2dd, 2H, J 9.0 Hz, J 5.1 Hz, J 13.8 Hz, $C^\beta H_2$ Tyr), 3.42, 3.56 (2dd, 2H, J 17.0 Hz, $C^\alpha H_2$ Gly), 4.33 (m, 1H, C^αH Tyr), 6.62, 6.98 (2m, 4H, C_6H_4 Tyr), 6.95 (t, 1H, NH Gly), 7.08, 7.38 (2s, 2H,

NH_2), 7.80 (d, 1H, J 8.1 Hz, NH Tyr), 9.17 (s, 1H, OH Tyr); $C_{16}H_{23}N_3O_5$.

General method for the synthesis of dipeptide amide trifluoroacetates I – VI.

A solution of 1 mmole of an N-*tert*-butyloxycarbonyl dipeptide in 10 ml of trifluoroacetic acid is stirred for 15 min at room temperature. Then the solvent is distilled off in vacuum and the residue is mixed with ether to obtain the target dipeptide amide trifluoroacetate with a quantitative yield in the form of a white crystalline substance.

***L*-Prolyl-*L*-tyrosine amide trifluoroacetate (*L*-Pro-*L*-Tyr-$NH_2 \cdot CF_3COOH$, I)**: m.p., 161 – 163°C; $[\alpha]_D^{20}$, – 13° (*c* 0.37; ethanol); R_f, 0.28 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.7 – 2.4 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.68, 2.92 (2dd, 2H, J 13.9 Hz, J 9.96 Hz, J 13.71 Hz, J 4.14 Hz, $C^\beta H_2$ Tyr), 3.07 – 3.25 (m, 2H, $C^\delta H_2$ Pro), 4.11 (m, 1H, $C^\alpha H$ Pro), 4.41 (m, 1H, $C^\alpha H$ Tyr), 6.65, 7.05 (2m, 4H, C_6H_4 Tyr), 7.14, 7.61 (2s, 2H, NH_2), 8.48 (s, 1H, NH Pro), 8.68 (d, 1H, J 8.1 Hz, NH Tyr), 9.31 (s, 1H, OH Tyr); $C_{14}H_{19}N_3O_3 \cdot CF_3COOH$.

***L*-Prolyl-*D*-tyrosine amide trifluoroacetate (*L*-Pro-*D*-Tyr-$NH_2 \cdot CF_3COOH$, II)**: m.p., 94 – 95°C; R_f, 0.26 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); $[\alpha]_D^{20}$, – 32° (*c* 2; chloroform); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.41 – 2.13 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.60, 2.99 (2dd, 2H, $C^\beta H_2$ Tyr), 3.11 (m, 2H, $C^\delta H_2$ Pro), 4.12 (m, 1H, $C^\alpha H$ Pro), 4.47 (m, 1H, $C^\alpha H$ Tyr), 6.62, 7.00 (2m, 4H, AA'XX' system, C_6H_4 Tyr), 7.18, 7.64 (2s, 2H, NH_2), 8.39, (s, 1H, NH Pro), 8.69 (d, 1H, NH Tyr), 9.17 (s, 1H, OH Tyr); $C_{14}H_{19}N_2O_3 \cdot CF_3COOH$.

***D*-Prolyl-*L*-tyrosine amide trifluoroacetate (*D*-Pro-*L*-Tyr-$NH_2 \cdot CF_3COOH$, III)**: m.p., 93 – 95°C; $[\alpha]_D^{20}$, + 32° (*c* 0.16; chloroform); R_f, 0.26 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.40 – 2.13 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.60, 2.99 (2dd, 2H, $C^\beta H_2$ Tyr), 3.12 (m, 2H, $C^\delta H_2$ Pro), 4.12 (m, 1H, $C^\alpha H$ Pro), 4.47 (m, 1H, $C^\alpha H$ Tyr), 6.63, 7.01 (2m, 4H, AA'XX' system, C_6H_4 Tyr), 7.20, 7.64 (2s, 2H, NH_2), 8.39, (s, 1H, NH Pro), 8.69 (d, 1H, NH Tyr), 9.17 (s, 1H, OH Tyr); $C_{14}H_{19}N_3O_3 \cdot CF_3COOH$.

***D*-Prolyl-*D*-tyrosine amide trifluoroacetate (*D*-Pro-*D*-Tyr-$NH_2 \cdot CF_3COOH$, IV)**: m.p., 155 – 156°C; $[\alpha]_D^{20}$, + 13° (*c* 0.4; ethanol); R_f, 0.28 (Kieselgel, butanol – acetic acid – water, 5 : 1 : 2); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.8 – 2.4 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.68, 2.92 (2dd, 2H, J 13.9 Hz, J 9.96 Hz, J 13.71 Hz, J 4.14 Hz, $C^\beta H_2$ Tyr), 3.07 – 3.25 (m, 2H, $C^\delta H_2$ Pro), 4.12 (m, 1H, $C^\alpha H$ Pro), 4.41 (m, 1H, $C^\alpha H$ Tyr), 6.64, 7.04 (2m, 4H, C_6H_4 Tyr), 7.14, 7.61 (2s, 2H, NH_2), 8.47 (s, 1H, NH Pro), 8.68 (d, 1H, J 7.86 Hz, NH Tyr), 9.31 (s, 1H, OH Tyr); $C_{14}H_{19}N_3O_3 \cdot CF_3COOH$.

Glycyl-*L*-tyrosine amide trifluoroacetate (Gly-*L*-Tyr-$NH_2 \cdot CF_3COOH$, V): m.p., 171 – 172°C; R_f, 0.37 (Silufol Kavalier, butanol – acetic acid – water, 5 : 1 : 2); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 2.64, 2.93 (2dd, 2H, J 9.8 Hz, J 4.6 Hz, J 13.9 Hz, $C^\beta H_2$ Tyr), 3.41, 3.61 (2d, 2H, J 16.5 Hz, $C^\alpha H_2$ Gly), 4.41 (m, 1H, $C^\alpha H$ Tyr), 6.65, 7.02 (2m, 4H, C_6H_4 Tyr), 7.13, 7.57 (2s, 2H, NH_2), 8.01 (s, 2H, NH_2 Gly), 8.59 (d, 1H, J 8.5 Hz, NH Tyr), 9.21 (s, 1H, OH Tyr); $C_{11}H_{15}N_3O_3 \cdot CF_3COOH$.

***L*-Prolyl-*L*-phenylalanine amide trifluoroacetate (*L*-Pro-*L*-Phe-$NH_2 \cdot CF_3COOH$, VI)**: m.p., 129 – 131°C; R_f, 0.54 (Silufol Kavalier, butanol – acetic acid – water, 5 : 1 : 2); $[\alpha]_D^{25}$ –18° (*c* 0.4; methanol); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.84 (m, 2H, $C^\delta H_2$ Pro), 1.84, 2.29 (2m, 2H, $C^\beta H_2$ Pro), 2.80, 3.04 (2dd, 2H, J 13.6 Hz, J 9.9 Hz, J 4.6 Hz, $C^\beta H_2$ Phe), 3.17 (m, 2H, $C^\delta H_2$ Pro), 4.10 (m, 1H, $C^\alpha H$ Pro), 4.47 (m, 1H, $C^\alpha H$ Phe), 6.99 – 7.40 (m, 5H, C_6H_5 Phe), 8.44, 9.34 (2s, 2H, NH_2), 8.74 (d, 1H, J 8.5 Hz, NH Tyr), no other signals below 20 ppm.

Synthesis of *L*-Prolyl-*L*-tryptophan amide trifluoroacetate (*L*-Pro-*L*-Trp-$NH_2 \cdot CF_3COOH$, VII).

(a) *tert*-Butyloxycarbonyl-*L*-proline N-hydroxysuccinimide ester (BOC-*L*-Pro-OSu). To a solution of 2 g (9 mmole) of BOC-*L*-Pro-OH and 1.07 g (9 mmole) of N-hydroxysuccinimide in 15 ml of dry THF at 0°C was added 2.11 g (10 mmole) of dicyclohexyl carbodiimide. The reaction mixture was kept for 2 h at 0°C and allowed to stand overnight in a refrigerator. Then the urea precipitate was filtered, THF distilled off in vacuum, and the residue crystallized from isopropyl alcohol to obtain. 2.79 g (94%) of BOC-*L*-Pro-OSu; m.p., 125 – 127°C; $[\alpha]_D^{25}$, – 55° (*c* 2; dioxane); R_f, 0.71 (Silufol Serva, dioxane – water, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.38 (s, 9H, $(CH_3)_3C$), 1.77 – 2.12 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 2.80 (s, 4H, $-(CH_2)_2-$), 3.35 (m, 2H, $C^\delta H$ Pro), 4.56 (m, 1H, $C^\alpha H$ Pro); $C_{14}H_{20}N_2O_6$. Published data: m.p., 135 – 136°C; $[\alpha]_D^{25}$, – 55.3° (*c* 2; dioxane) [19].

(b) *tert*-Butyloxycarbonyl-*L*-prolyl-*L*-tryptophan (BOC-*L*-Pro-*L*-Trp-OH). A solution of 2 g (6.4 mmole) of BOC-*L*-Pro-Osu in 10 ml of DMF was cooled to 0°C and added to a mixture of 1.36 g (6.7 mmole) of *L*-tryptophan and 2 ml of 3.5 M KOH in 10 ml DMF at 0°C. The reaction mixture was kept for 1 h at 0°C and allowed to stand overnight at room temperature. Then the solvent was distilled off in vacuum and the residue dissolved in chloroform and washed with 0.01 N HCl. The organic layer was separated, washed with water, and dried over $MgSO_4$. Finally, chloroform was distilled off in vacuum to obtain 2.27 g (88%) of BOC *L*-Pro-*L*-Trp-OH in the form of crystallizing oil; R_f, 0.4 (Silufol Serva, chloroform – methanol, 9 : 1); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.16, 1.37 (2s, 9H, $(CH_3)_3C$), 1.6 – 2.1 (m, 4H, $C^\beta H_2 - C^\gamma H_2$Pro), 3.0 – 3.4 (m, 2H, $C^\beta H_2$), 3.4 (m, 2H, $C^\delta H_2$ Pro), 4.06 – 4.17 (m, 1H, $C^\alpha H$ Pro), 4.43 – 4.49 (m, 1H, $C^\alpha H$ Trp), 6.9 – 7.6 (m, 5H, H_{arom}, CH=), 7.96, 8.01

(2d, 1H, J 7.0 Hz, J 7.7 Hz, NH Trp), 10.84, 10.87 (2s, 1H, NH Trp ring), 12.58 (s, 1H, OH); $C_{21}H_{27}N_3O$.

(c) *tert*-Butyloxycarbonyl-*L*-prolyl-*L*-tryptophan amide (BOC-*L*-Pro-*L*-Trp-NH_2). To a solution of 1.87 g (4.67 mmole) of BOC-*L*-Pro-*L*-Trp-OH in 16 ml of DMF cooled to −10°C was added dropwise with stirring 0.59 ml (4.67 mmole) of N-ethylmorpholine and 0.605 ml (4.67 mmole) of isobutylchloroformate. After 1 – 2 min, to this mixture was slowly added 20 ml DMF saturated with gaseous ammonia. The reaction mixture was kept for 1 h at 10°C and for 30 min at room temperature. The precipitate was separated by filtration, the filtrate evaporated, and the residue dissolved in chloroform. The solution was successively washed with 5% $NaHCO_3$, water, and 1 N HCl, and dried over $MgSO_4$. Finally, the solvent was distilled off in vacuum to obtain 1.47 g (79%) of a chromatographically homogeneous product in the form of a yellowish oil; R_f, 0.4 (Silufol Serva, chloroform – methanol, 9 : 1); $[\alpha]_D^{20}$, −62.7° (*c* 0.4; methanol); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.18, 1.35 (2s, 9H, $(CH_3)_3C$), 1.55 – 2.10 (m, 4H, $C^\beta H_2$ – $C^\gamma H_2$Pro), 2.9 – 3.3 (m, 4H, $C^\alpha H_2$ Trp + $C^\delta H_2$ Pro), 4.03 (m, 1H, C^αH Pro), 4.41 – 4.50 (m, 1H, C^αH Trp), 6.44 (s, 1H, NH_2), 6.9 – 7.9 (m, 8H, H_{arom}, CH=, NH_2, NH), 7.67, 7.82 (2d, 1H, J 8.0 Hz, NH), 10.78, 10.82 (2s, 1H, NH Trp); $C_{21}H_{28}N_4O_4$.

(d) *L*-Prolyl-*L*-tryptophan amide trifluoroacetate (*L*-Pro-*L*-Trp-$NH_2 \cdot CF_3COOH$, VII). A solution of 0.3 g (0.724 mmole) of BOC-*L*-Pro-*L*-Trp-NH_2 in 2 ml of methylene chloride was cooled to 0°C and added to a mixture of 6 ml CF_3COOH, 3 ml of methylene chloride, and 0.15 ml of mercaptoethanol at 0°C. The reaction mixture was kept for 30 min at room temperature and evaporated. Finally, the residue was mixed with ether to obtain 0.17 g (76%) of *L*-Pro-*L*-Trp-$NH_2 \cdot CF_3COOH$; m.p., 96 – 103°C; R_f, 0.2 (Silufol Serva, butanol – acetic acid – water, 5 : 1 : 2); $[\alpha]_D^{20}$, −15.5° (*c* 0.4; methanol); ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 1.87, 2.29 (2m, 2H, $C^\beta H_2$ Pro), 1.87 (m, 2H, $C^\gamma H_2$ Pro), 2.97, 3.31 (2dd, 2H, $C^\beta H_2$ Trp), 3,19 (m, 2H, HDO, $C^\delta H_2$ Pro), 4.11 (m, 1H, C^αH Pro), 4.53 (m, 1H, C^αH Trp), 6.9 – 7.8 (m, 7H, H_{arom}, CH=, NH_2), 8.7 (d, 1H, NH Pro), 10.86, 10.87 (2s, 1H, NH Trp); $C_{18}H_{21}N_4F_3O_4$.

EXPERIMENTAL PHARMACOLOGICAL PART

Inhibition of the apomorphine-induced verticalization in mice. Experiments were performed on white mongrel male mice weighing 22 – 25 g. The compounds to be tested were suspended in an 0.9% aqueous NaCl solution containing 2.5% of Tween-80 and introduced intraperitoneally 15 min prior to apomorphine injections. Apomorphine was introduced subcutaneously at a dose of 5 mg / kg with an 9% NaCl solution containing 0.1% of ascorbic acid. Animals in the apomorphine-treated control group were injected with physiological solution. Intact control group was also injected with the physiological solution.

After the apomorphine introduction, a test animal was placed in a 12-cm-diam 14-cm-high wire cylinder. After a 15-min period, the verticalization parameters were monitored every 2 min during a 1-h observation time. The results were assessed against the following scale: rate 0, all four paws on the floor (no verticalization effect); rates 1 – 3, one to three paws on the cylinder wall, respectively; rate 4, all four paws on the wall (full verticalization). The total sum of data obtained within one hour in the control was taken as 100%. The data were statistically processed according to the Wilcoxon – Mann – Whitney method.

Recovery of extrapolated avoidance reflex in rats upon the madopar induced violation. Experiments were performed on white mongrel male rats (obtained from the Stolbovaya nursery, Moscow oblast) weighing 250 – 280 g. The test setup was a glass bottomless cylinder provided with a removable nontransparent cover. The cylinder edge was immersed to 2 cm in water (the total depth of water in a vessel, 20 cm; water temperature, 22°C). One day before testing, all animals were placed in the cylinder for 2 min for the training. The compounds to be tested were dissolved in a physiological solution containing 2.5% Tween-80 and injected subcutaneously to the animals 70 min prior to the test. Ten minutes later, the animals were injected subcutaneously with a madopar suspension (100 mg / kg *L*-DOPA + 25 mg / kg benzerazide in a physiological solution containing 2.5% Tween-80). Every animal tested was placed for 2 min in the cylinder and the number of animals diving under the cylinder edge to escape from the stressful situation was determined in each group. The percentage activity was calculated by the formula

$$\text{Activity} = \frac{(N_{exp} - N_{contr})}{(N_{int} - N_{contr})} \times 100\%,$$

where N_{exp}, N_{contr}, and N_{int} are the numbers of animals solving the task in a given experimental group, in the control group, and in the intact group. The experimental results were statistically processed using the Fisher criterion.

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