

Boc-Protected Proline-Containing Monoamines as Promising Prodrugs

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Abstract: In recent years, drug designers have increasingly focused on creating complex compounds containing biologically active molecules of various chemical classes in their structure. Such modifications increase the stability of resulting molecules in the organism and increase their ability to achieve the desired targeting effect. Regulatory peptides-based drugs are of particular interest. In this work, we combined the monoamines (serotonin or dopamine) and *N*-terminal-protected biologically active peptides or amino acids in a single compound and characterized the resulting molecules' enzymatic stability and possible molecular activity. Boc-Pro-5HT and Boc-Pro-DA were stable for at least 180 min of incubation in the presence of PEP, amino- and carboxy-peptidases, as well as blood plasma. This time is enough to reach their corresponding biological targets in the organism and become additional monoamines and regulatory peptides. It was also shown that these complex compounds possess specific targets on cell plasmatic membranes and can affect some neuroreceptor systems (GABA and Ach, in particular) as highly selective allosteric modulators. The above features make these molecules promising candidates for prodrugs and require further research on the mechanisms of their action.

Keywords: prodrugs, monoamines, Boc-Pro-5HT, Boc-Pro-DA, degradation, specific binding, GABA, Ach, peptides, Boc-protection, radioligands

Abbreviations: 5HT – Serotonin; DA – Dopamine; ACh – Acetylcholine; GABA – Gamma-Aminobutyric acid (γ -aminobutyric acid); Dox – Doxorubicin; l-DOPA – Dopamine-precursor levodopa; TRP – Tryptophan; PEP – Prolyl Endopeptidase; Et3N – Triethylamine; DMF – Dimethylformamide; DCC – Dicyclohexylcarbodiimide; DCHC – 3-(2,4-Dichlorophenyl)-7-hydroxy-4H-chromen-4-one; EDTA – Ethylenediaminetetraacetic acid; PMSF – phenylmethylsulfonyl fluoride; Tris – Tris(hydroxymethyl)aminomethane; TFA – Trifluoroacetic acid; Boc – Tert-butyloxycarbonyl protecting group; Z (or Cbz) – Benzyloxycarbonyl protecting group; Fmoc – Fluorenylmethoxycarbonyl protecting group; PBS – Phosphate-buffered saline; BSA – Bovine serum albumin; BBB – Blood-brain barrier; PAMPA – Parallel Artificial Membrane Permeability Assay; HPLC – High-performance liquid chromatography; PD – Parkinson's disease; K_d – (radioligand) equilibrium dissociation constant; B_{max} – Total density (concentration) of receptors in a sample of tissue.

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1. Introduction

Monoamine signaling deficiency is a well-known mechanism of Parkinson's disease (PD) [1, 2, 3] and depression [4, 5]. The above diseases can often occur together, and patients with atypical PD more commonly have depression than patients with classical PD presentations [6, 7, 8, 9]. As a rule, neurotransmitters such as dopamine, serotonin, and norepinephrine are all depleted in PD [10, 2]. The above neurotransmitters work in conjunction and indirectly affect each other [11]. Normally, serotonin and dopamine affect the neural circuits of serotonergic, noradrenergic, and dopaminergic neurons, which in turn regulate mood, motivation, and other cognitive functions [12, 13]. The loss of dopaminergic neurons characterizes the neuropathology of PD. Still, the neurodegeneration in PD extends far beyond the dopaminergic neurons. It is found in noradrenergic and serotonergic neurons and other neurons located in the cerebral prefrontal cortex, olfactory bulb, and other regions of the brain [14, 15]. In addition to the above, the metabolism of tryptophan (TRP, a precursor of serotonin [16]) in PD and depression may be shifted from the synthesis of serotonin to other, potentially neurotoxic compounds, such as the tryptophan catabolite, or quinolinic acid [17, 18].

For a safe and effective treatment, or for mild prevention of disease and/or depression, we need (ideally) a drug with the following desirable properties: mild action, strong regulation effect, good stability in the body, easy delivery to its target, and safe elimination or biodegradation. In recent years, drug designers have increasingly focused on creating complex compounds containing biologically active molecules of various chemical classes in their structure. Regulatory peptides-based drugs are of particular interest [19, 20, 21]. For example - arachidonoyl-semaphorin [22], docosahexanoyl-PGP, and other biologically active peptolipins (compounds based on peptides and neuroactive fatty acids); PGP-Dopa, and others. Compound molecules may possess biological activities that differ from the effects of the components that make up their structure [23, 24, 25, 26, 27, 28]. This means that in the case of a successfully created molecule, we obtain a compound that first acts by itself, and then its components (products of directed biodegradation of the original molecule) work. Unfortunately, not all complex compounds that are effective “*in vitro*” are characterized by a sufficiently high bioavailability, which is necessary, for example, to overcome the blood-brain barrier, or by sufficiently high resistance to blood enzymatic activity. Commonly, peptide-based structures undergo fast proteolytic degradation of their *N*- and *C*-terminal sides by several endopeptidases. Therefore, it is important to synthesize structures with terminal bonds resistant against fast deactivation [29]. It has recently been shown that the introduction of (Boc)- and (Z)- blocked amino acids into a peptide structure allows to increase the stability of peptide-based molecules as well as to increase their ability to achieve desired targeting effect [30, 31, 32]. In this work, we combined the monoamines (serotonin and dopamine) and *N*-terminal protected peptides (and amino acids) into single compounds and characterized the enzymatic stability and possible molecular activity of the resulting molecules.

2. Materials and Methods

2.1. Reagents.

Catalysts, reagents, and solvents are commercial preparations. All the Boc-protected Pro derivatives of dopamine and serotonin and tritium-labeled ligands were synthesized at the National Research Centre «Kurchatov Institute» - Institute of Molecular Genetics (Russia).

Eppendorf pipettes and plastic plates were used. The radioligand-receptor analysis was carried out on a special device; the 96-well filter plates MultiScreenHTS (Multiscreen system, EMD Millipore, Darmstadt, Germany) were used.

2.2. Buffers.

Buffer A (10 mM Tris-HCl, pH 7.4 at 4 °C, saccharose 0.32 M, 1 mM EDTA, 1 mM benzamidine, 0.1 mM PMSF); Buffer A2 (10 mM Tris-HCl, pH 7.4 at 4 °C, 0.22 M saccharose); Buffer B (50 mM Tris-HCl, 1 mM CaCl₂, 0.003% BSA, pH 7.4 at 30 °C - for incubation; or pH 7.4 at 4 °C - for plates washing).

2.3. Animal management.

We used male adult albino outbred rats with an average body weight of 180–200 g. The rats were kept in plastic cages during all the experiments under standard laboratory conditions: a 12 h light/dark cycle, a controlled ambient temperature (22–25 °C), and 60% ± 10% humidity. Animals from all groups (before the start of an experiment) were allowed access to standard laboratory rat pellet chow and water ad libitum. All experimental animal procedures were approved by the Experimental Animal Care Society (National Research Centre «Kurchatov Institute» - Institute of Molecular Genetics, Moscow).

2.4. Isolation of plasma membranes and blood plasma.

All plasma membrane isolation procedures were performed at 4°C. Rats were decapitated, their brains were extracted and washed with cold PBS. In Buffer A were extracted cortex and hippocampus. The extracted samples were homogenized in 10 volumes of Buffer A, and centrifuged at 1000 g for 20 min. The sediment was removed, and the supernatant was centrifuged again at 40000 g for 30 min. The mitochondria-rich dense brown sediment at the bottom of the tube was removed. The light sediment of membranes was transferred to a clean tube and resuspended (washed) in Buffer A. After the second centrifugation at 40000 g for 30 min, the sediment was resuspended in Buffer A2. Further, according to the Hartree–Lowry method, the obtained samples determined the protein content. The membranes were divided into portions, frozen in liquid nitrogen, and stored at -70 °C (no longer than 30 days).

Blood (5 mL) was collected in a test tube containing 20 µL of heparin (5000 U/mL). After centrifugation at 1500 g for 10 min, the protein concentration (of 31 mg protein/mL) was determined (by the Hartree-Lowry method).

2.5. Radioligand binding.

The tritium-labeled ligands -[³H]GABA and [³H]Ach were synthesized at the National Research Centre «Kurchatov Institute» - Institute of Molecular Genetics, Russia. After the introduction of tritium, isolation and purification were obtained: [³H]GABA with molar radioactivity of 52 Ci/mmol and radiochemical purity > 95% [33] and [³H]Ach with molar radioactivity of 30 Ci/mmol and radiochemical purity > 97% [34].

The reaction mixture (final volume, 200 µL) was incubated in the standard 96-well plates with GF/B filters. Each well contained 50 µL of the labeled ligand (in Buffer B), 50 µL of unlabeled ligand or the compound under study (in Buffer B), and 100 µL of membrane protein solution. Membranes (whose final concentration in the incubation mixture was 0.2

mg/mL) were dissolved in Buffer B with an addition of inhibitors (100 μ M PMSF + 10 μ M Bacitracin + 5 μ M Pepstatin A). After 20 min of incubation (at 30 °C with continuous shaking), the samples were passed straight through the filters at the bottom of the plates and immediately washed with three portions of cold Buffer B (200- μ L each). The plates were air-dried; filters were detached and transferred into scintillation vials. We used 4 mL of the liquid scintillator (Unisolve 100; Koch-Light, Haverhill, UK). The radioactivity was measured using a Tri-Carb 2100R liquid scintillation counter (Packard BioScience, USA). We used SigmaPlot 10.0 (Systat Software, San Jose, CA) for the mathematical processing of the obtained results.

2.6. Synthesis of Boc-Pro-5HT and Boc-Pro-DA.

The initial compounds and reaction products were characterized using high-performance liquid chromatography (HPLC) and mass spectrometry. Mass spectrometric data were obtained using an LCQ Advantage MAX device (Thermo Electron Corp., USA), with electrospray ionization, direct injection of a sample solution with a concentration of 10 μ g/ml in 0.1% acetic acid, and further fragmentation of the molecular peak in the analyzer by ion collisions at 35 eV. The synthesis was carried out according to the methods [35, 36, 20].

To obtain a serotonin derivative with proline (Pro-5HT) and a dopamine derivative with proline (Pro-DA), Boc-Pro was chosen as the proline component. Condensation was carried out by the traditional method using dicyclohexylcarbodiimide (DCC). A solution of 33.3 mg (0.15 mmol) of Boc-Pro, 21.5 mg (0.16 mmol) of 1-oxybenztriazole, 35 mg (0.17 mmol) of DCHC in 2 mL of dimethylformamide (DMF) and 150 μ L of triethylamine (Et_3N) was added with 30 mg (0.14 mmol) of HCl-5HT (or HCl-DA). The reaction was carried out for 12–17 hours, and the DMF was removed by lyophilization. The reaction mixture was analyzed on a ProntoSIL column-120-5-C18 AQ DB-2003 ("Bishcoff Chromatography", Germany, size 2.0 $\times$ 75 mm, particle diameter 5 microns), in system A (0.1% CH_3COOH), in system B (methanol), gradient B - from 30 to 100% in 12.5 min, eluent feed rate-0.2 mL/min. Boc-Pro-5HT holding time-5.06 min. Boc-Pro-DA was holding time- 4,69 min. Boc-Pro-5HT (Boc-Pro-DA the same) was isolated by solid-phase extraction on a Diapak C16 cartridge (Granat, Russia). The substance was extracted from the carrier with aqueous methanol with 0.05% acetic acid, gradually increasing the methanol content from 20 to 100% (it is recommended to apply no more than 5 mg of the reaction mixture per 1 g of the phase). Preparative chromatography was performed on a Reprosil pur C18aq column ("Dr. Maisch GmbH", Germany, size 20 $\times$ 150 mm, particle diameter 10 microns), in a system of 50% methanol + 0.05% acetic acid, eluent feed rate-20 mL/min, wavelength 280 nm. The yield of Boc-Pro-5HT was in the range of 75-80%, Boc-Pro-DA – 65-80%.

The enzymatic stability of Boc-Pro-5HT and Boc-Pro-DA in amino- and carboxypeptidases, as well as PEP and rat blood plasma presence, was studied according to [37].

The Leucine Aminopeptidase (EC3.4.11.2), Carboxypeptidase Y (EC3.4.16.1), Carboxypeptidase B (EC3.4.17.2), and Prolyl Endopeptidase (PEP) (EC3.4.21.26) were used. The following ratios were used: 0.092 μ mol/U - for carboxypeptidase Y; 1 μ mol/U - for leucine aminopeptidase; 0.022 μ mol/U - for carboxypeptidase B; 0.10 μ mol/U - for PEP. Enzymatic hydrolysis of proline derivatives (of dopamine and serotonin) was carried out in PBS (27.4 mM NaCl, 0.4 mM KCl, 2 mM Na_3PO_4 in 100 mL H_2O , pH 7.4) according to the following procedure. A solution of 0.5 μ mol of a proline-containing derivative in 260 μ L of PBS was

treated with an enzyme (0.50 U of leucine aminopeptidases, or 22.46 U of carboxypeptidase B, or 5.0 U of PEP, or 5.45 U of carboxypeptidase Y) and stirred and thermostated at 30°C. The aliquots (20 µL) were taken at certain time intervals. The hydrolysis was stopped by adding an equal volume of methanol.

For the blood plasma, 820 µL of a peptide derivative solution (0.74 µmol in PBS) was mixed with 380 µL of blood plasma (31 mg protein/mL) and stirred and thermostated at 30 °C. The aliquots (150 µL) were taken at certain time intervals. The peptide fraction was purified with a reverse-phase Lichroprep RP-18, followed by elution of the peptides with methanol with 0.1% TFA. Then, the mixture was evaporated and dissolved in methanol: water (5 : 95) solution (150 µL) and analyzed by HPLC.

3. Results

3.1. Enzymatic stability of Boc-Pro-5HT and Boc-Pro-DA.

The proline-containing monoamines Boc-Pro-5HT and Boc-Pro-DA showed high stability for at least 180 min of incubation in the presence of amino- and carboxy-peptidases, as well as blood plasma enzymes (Table 1). The stability of Boc-Pro-5HT and Boc-Pro-DA in PEP presence was determined at the ratios of Boc-Pro-5HT and Boc-Pro-DA to PEP 0.1 µmol / unit (Figure 1).

Table 1. The stability of Boc-Pro-DA and Boc-Pro-5HT in the presence of amino-, carboxypeptidases and blood plasma enzymes.

Substrate	Time, min					
	10	60	180	240	1440	4320
Leucine aminopeptidase						
Boc-Pro-DA	100.00*	98.83	95.01	91.79	73.61	69.50
Boc-Pro-5HT	100.00	99.16	95.54	91.92	88.30	81.06
Carboxypeptidase Y						
Boc-Pro-DA	94.08	90.42	88.73	87.89	76.90	74.37
Boc-Pro-5HT	98.46	91.92	86.92	85.77	80.77	79.23
Carboxypeptidase B						
Boc-Pro-DA	97.84	93.83	89.20	84.57	79.94	73.15
Boc-Pro-5HT	93.99	81.98	80.92	79.86	78.80	77.39
Blood Plasma						
Boc-Pro-DA	100.00	99.16	98.60	98.04	93.02	86.87
Boc-Pro-5HT	99.76	99.52	99.28	98.80	94.46	89.16

* the content of the initial compound in the incubation solution, %.

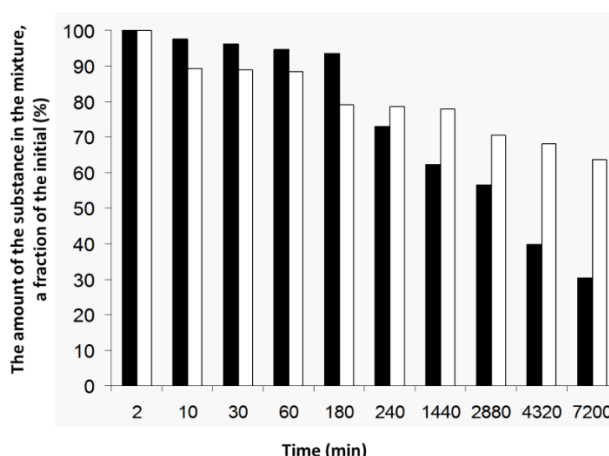


Figure 1. The stability of Boc-Pro-5HT and Boc-Pro-DA in the presence of PEP.

A substrate-to-PEP ratio is 0.1 μmol / unit; black column – Boc-Pro-DA; white column – Boc-Pro-5HT.

3.2. [^3H]GABA and [^3H]ACh specific binding.

The tritium-labeled ligands were characterized by molar radioactivity of 52 Ci/mmol and radiochemical purity of > 95% for [^3H]GABA and 30 Ci/mmol and > 97% for [^3H]ACh. The composition of incubation buffers, incubation temperature and time, as well as the combination of inhibitors that we used for the radioligands, were also found to be optimal for incubation of Boc-Pro-5HT and Boc-Pro-DA - there was no active biodegradation, chemical modification, or other changes in the primary structure of the studied molecules. The sites of [^3H]GABA and [^3H]ACh specific binding was characterized on rat cortical cells plasmatic membranes, the constants of specific binding and the values of the maximum number of specific binding sites (K_d and B_{max} , respectively) were calculated. The characteristics of the radioligands binding sites were consistent with the previously obtained values [38, 39], which allows us to compare the previous results with the data of current experiments.

3.3. The influence of Boc-Pro-5HT and Boc-Pro-DA on [^3H]GABA and [^3H]ACh specific binding.

The following sites were studied: the high-affinity site of [^3H]GABA binding, characterized by K_d -15 nM and B_{max} -60 pmol/g of membrane protein; and two binding sites [^3H]ACh - one high-affinity, characterized by K_d - 4 nM and B_{max} - 2 pmol/g of membrane protein and one low-affinity, characterized by K_d - 100 nM and B_{max} - 18 pmol/g of membrane protein, respectively. We studied the influence of Boc-Pro-5HT and Boc-Pro-DA on radioligand binding within an ultra-wide range of their (monoamines) concentrations: from femto- Mol (10–15 mol per liter) to micro- Mol (10–6 mol per liter).

The data is presented as a diagram in % of the radioligand specific binding (Y-axis) and logarithmic concentration of an effector, mol per liter (X-axis). The specific binding of the radioligand accepts as 100% (a value of 100% corresponds to specific binding, determined in the presence of unlabeled ligand), and the binding in effector presence is recalculated relative to the % of specific.

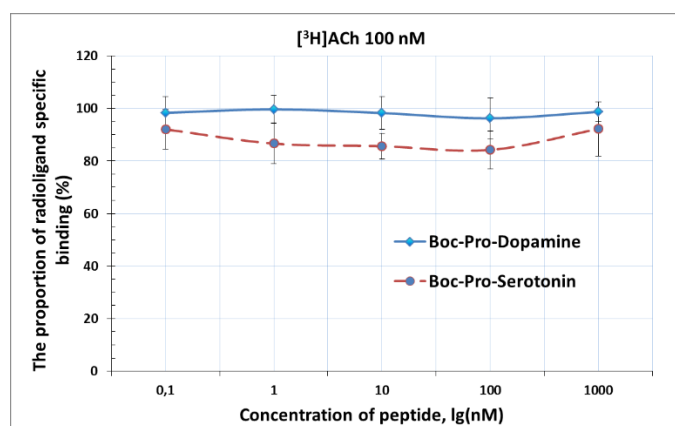


Figure 2. Binding of [^3H]ACh to corresponding low-affine sites on rat cerebral cortex cells plasmatic membranes in the presence of Boc-Pro5HT and Boc-Pro-DA.

The binding of [^3H]ACh to corresponding receptors on rat cerebral cortex cells plasmatic membranes in the presence of Boc-Pro-5HT and Boc-Pro-DA is shown in Figure 2

and Figure 3. The data obtained indicate no significant effect of Boc-Pro-monoamines on [3 H]ACh specific binding at its low-affinity site ($K_d = 100$ nM). At the same time, the Boc-Pro-monoamines were active against the high-affinity site of [3 H]ACh (Figure 3). The study of [3 H]GABA binding in Boc-Pro-monoamines presence showed the difference between their effects. Both derivatives showed no effect at their low concentrations, but at concentrations of 10 nM and higher, Boc-Pro-DA has a slight blocking effect on [3 H]GABA binding, not exceeding 20% (Figure 4).

The abscissa (logarithmic) represents the concentration of the monoamine derivative in the incubation mixture, nmol per liter (nM). The ordinate represents the proportion of [3 H]ACh (100 nM) specific binding, %. A value of 100% corresponds to [3 H]ACh specific binding, determined in the presence of unlabelled ACh.

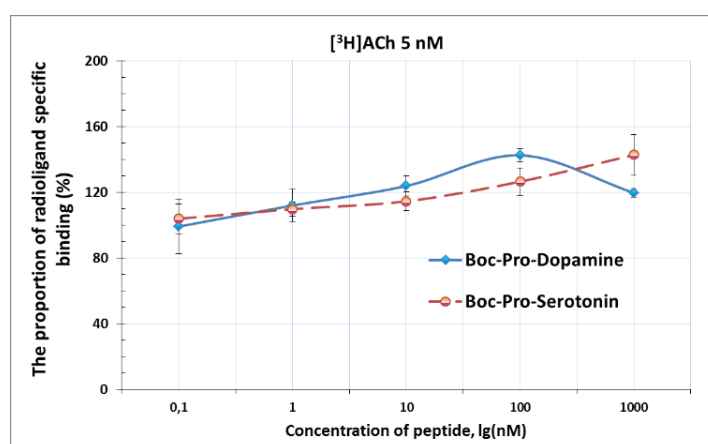


Figure 3. Binding of [3 H]ACh to corresponding high-affine sites on rat cerebral cortex cells plasmatic membranes in the presence of Boc-Pro-5HT and Boc-Pro-DA.

The abscissa (logarithmic) represents the concentration of monoamine derivative in incubation mixture, nmol per liter (nM). The ordinate represents the proportion of [3 H]ACh (5 nM) specific binding, %. A value of 100% corresponds to [3 H]ACh specific binding, determined in the presence of unlabelled ACh.

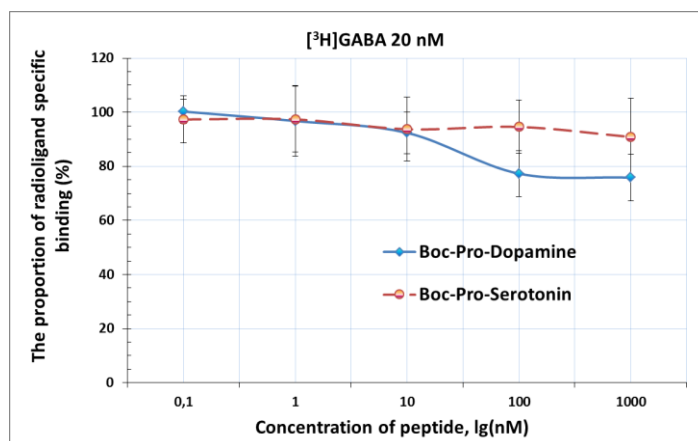


Figure 4. Binding of [3 H]GABA to corresponding receptors on rat cerebral cortex cells plasmatic membranes in the presence of Boc-Pro-5HT and Boc-Pro-DA.

The abscissa (logarithmic) represents the concentration of monoamine derivative in incubation mixture, nmol per liter (nM). The ordinate represents the proportion of [3 H]GABA (20 nM) specific binding, %. A value of 100% corresponds to [3 H] GABA-specific binding, determined in the presence of unlabelled GABA.

4. Discussion

N-Boc/Fmoc/Z-blocked peptide derivatives are the intermediates particularly useful in synthesizing partially modified peptides [40, 41, 42]. Such modifications can also extend the lifetime of peptide-based structures in the living organism, which increases their ability to achieve the targeting effect [30]. Previously, we synthesized Z- and Boc- protected structures based on the Gly-Pro dipeptide and analogs of endogenous neurotransmitters - dopamine and serotonin (Z-Gly-Pro-DA, Z-Gly-Pro-5HT, Boc-Gly-Pro-DA, Boc-Gly-Pro-5HT). The above compounds were shown to be stable in the presence of leucine aminopeptidase, carboxypeptidase Y, and carboxypeptidase B but hydrolyzed to release dopamine and serotonin in the presence of prolyl endopeptidase [37]. It was also shown that compounds containing Boc- protection are more stable among the above structures than derivatives containing Z- protection [37].

In addition to low enzymatic stability, another significant barrier to the path of the active substance to its target in the brain is the blood-brain-barrier (BBB). It is known that many peptides and peptide-containing derivatives of biologically active compounds are able to penetrate the BBB by passive diffusion [43]. To evaluate this ability for the above peptide-containing derivatives, we used artificial membranes, according to PAMPA (Parallel Artificial Membrane Permeability Assay) method [44]. A comparison of Z - and Boc- protected variants and structures based on the Gly-Pro dipeptide and only Pro showed that the most promising in overcoming the BBB by passive diffusion is Boc- protected Pro-containing structures [45]. Therefore, we used Boc-protected glycine-free compounds (Boc-Pro-5HT and Boc-Pro-DA) in this work.

The above molecules were stable for at least 180 min of incubation in the presence of amino- and carboxypeptidases (Table 1), as well as for at least 60 min in the presence of high concentrations of PEP (Figure 1). Compared to Gly-Pro dipeptide derivatives (Boc-Gly-Pro-DA and Boc-Gly-Pro-5HT) [37], the stability of proline-containing derivatives of dopamine and serotonin was quite higher, even with a higher PEP ratio (0.1 $\mu\text{mol/u}$). Let's compare the stability of proline-containing derivatives of dopamine and serotonin with each other. It can be stated that initially, Boc-Pro-DA has a certain stability advantage, but after a few hours, its degradation accelerates and exceeds the corresponding values of Boc-Pro-5HT degradation (Figure 1). It is interesting to note that during the first 240 min of incubation of Boc-Pro-DA and Boc-Pro-5HT in the presence of PEP, in contrast to Boc-Gly-Pro-DA and Boc-Gly-Pro-5HT, the release of free dopamine and serotonin does not occur. The molecules are also stable in blood plasma (0.063 $\mu\text{mol/mg}$ protein) for at least 240 min (Table 1). The data obtained suggest that the above-mentioned proline-containing monoamines are able to reach the corresponding biological targets unchanged and, depending on the local enzymatic activity, become an additional source of monoamines.

In particular, complex molecules based on peptides may possess their biological activity, different from their constituent compounds. For example, experiments using a test system based on *Tetrahymena pyriformis* infusoria showed that peptide modification of the anthracycline antibiotic Doxorubicin reduces cytotoxic and cytostatic properties unmodified Dox as well as changes cell motility [46]. It is also known that regulatory peptides and neuropeptides are characterized by a wide spectrum of allosteric activity against several different neuroreceptor systems [38, 39], and the peptide-containing derivatives of biologically

active molecules possess their specific targets on plasmatic membranes of brain cells [22]. The studied compounds may also have their unique spectrum of molecular activity.

To evaluate the existence of the effect mentioned above in Boc-Pro-5HT and Boc-Pro-DA molecules, we used a radioligand-receptor method of analysis. The Acetylcholine and GABA receptor systems were selected as potential targets. The above receptor systems are widely represented in different organs and tissues and are one of the most important regulators of brain nerve cells activity. In addition, the receptors of these subtypes are not the targets of serotonin and dopamine, which excludes the influence of the above components of complex molecules on the results obtained. We tested the effect of Boc-Pro-5HT and Boc-Pro-DA on [^3H]GABA and [^3H] Ach-specific binding. The following sites were studied: the high-affinity site of [^3H]GABA binding, characterized by K_d 15 nM, and two binding sites [^3H]Ach - one high-affinity and one low-affinity, characterized by K_d 4nM and 100 nM, respectively. The range of Boc-Pro-5HT and Boc-Pro-DA concentrations covered the traditional therapeutic area (micro-Molar) and the areas of low and ultra-low concentrations (pico- and nano-Molar). A single radioligand concentration corresponding to the affinity of one particular specific site of binding was used for incubation with plasmatic membranes. The value of the radioligand-specific binding on the selected site was assumed to be 100%. The values obtained during incubation of the radioligand in the presence of different concentrations of the test compound were expressed as fractions of the specific binding of the radioligand. It is important to note that allosteric effects of Boc-Pro-5HT and Boc-Pro-DA were quite difficult to detect and characterize. Thus, it was assumed that only changes with values of at least 15–20 units (% of specific radioligand binding) are statistically significant.

The compounds (Boc-Pro-5HT and Boc-Pro-DA) did not significantly affect the binding of [^3H]Ach at its low-affinity site (Figure 2), as well as the binding of [^3H]GABA at its high-affinity site (Figure 4). Note that at concentrations of 100nM and higher, Boc-Pro-5HT was inactive in relation to [^3H]GABA binding, but Boc-Pro-DA reduced binding by about 20%. A pronounced concentration-dependent effect was shown for the low-affinity site of [^3H]Ach (Figure 3). With an increase in the concentration of Boc-Pro-5HT by one order of magnitude, the binding of [^3H]Ach increased by approximately 10%. Boc-Pro-DA showed similar but not the same effects (Figure 3). All the above indicates a high probability that the studied compounds have their own receptor-specific targets on cellular plasma membranes, selectively affect certain neuroreceptor systems functional activity, and, consequently, may possess their own biological effects different from that of their constituent compounds.

5. Conclusions

The observed data showed that the studied Pro-containing monoamines protected by (Boc-) are stable enough to reach their corresponding biological targets unchanged and, depending on the local enzymatic activity, become an additional source of monoamines. It was also shown that these compounds possess their own specific targets on cell plasmatic membranes and can affect some neuroreceptor systems as selective allosteric modulators. The above features make Boc-Pro-containing monoamines promising candidates for creating prodrugs and require further research on the mechanisms of their action.

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Conflicts of Interest

The authors declare no conflict of interest.

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