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SYNTHESIS AND NOOTROPIC ACTIVITY PREDICTION OF SOME 4-(AMINOMETHYL)-1-BENZYLPYRROLIDIN-2-ONE DERIVATIVES STRUCTURALLY RELATED WITH NEBRACETAM

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The aim. *Search for new biologically active substances with improved nootropic parameters among analogues of 4-(aminomethyl)-1-benzylpyrrolidine-2-one (Nebracetam).*
Materials and methods. *The required reagents were purified using standard techniques. The elemental analysis was performed on a "Hewlett Packard" automatic analyzer M-180 company. ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz spectrometer in DMSO-d6 as a solvent. LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped. The Autodock 4.2 software package was used for molecular docking. The active centers of the peptides (PDB ID: 5CXV, 6PV7) was used as the biologycal targets.*
Results and discussion. *Basic and alternative methods (1 and 2) of obtaining were used to synthesise target analogues of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one. As a result of synthetic studies, an optimized method with an alternative method has been proposed. The advantages include reducing the duration and number of synthesis stages and avoiding the use of sodium azide, a highly toxic and hazardous substance. Molecular docking of the synthesized compounds at well-documented acetylcholine receptor sites indicates that all tested molecules will contribute to the manifestation of nootropic activity to varying degrees through cholinergic neurotransmission mechanisms. This is evidenced by the calculated docking values in relation to the muscarinic target. According to the docking results, it was found that depending on the enantiomeric configuration, the molecules formed stable complexes with the target and had characteristic binding modes both in the orthosteric site and in the extracellular vestibule (site of positive allosteric modulation of mAChR). It indicates the prospects of modifying the "nebracetam scaffold" at the phenyl fragment with halogen substituents.*
Conclusions. *An effective method for synthesising analogues of 4-(aminomethyl)-1-R-benzylpyrrolidin-2-ones has been developed. The molecular docking revealed potential mechanisms of nootropic action of the synthesized derivatives as potential agonists and positive allosteric modulators of the muscarinic receptor*
Keywords: *synthesis, 4-(aminomethyl)-1-benzylpyrrolidin-2-one, molecular docking, acetylcholine receptors, nootropic activity*

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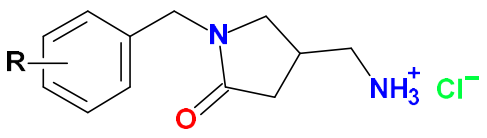
1. Introduction

It is advisable to search for new chemical structures that can exhibit a specific biological effect in the class of chemical compounds where substances with a certain direction of action have already been detected. The group of pyrrolidone derivatives (2-oxopyrrolidine) has been the research subject for more than three decades. The five-membered pyrrolidine ring is widely used as a base scaffold for obtaining one of the promising groups of psychoactive nootropic agents of the racetam group (piracetam, aniracetam, oxiracetam, pramiracetam, phenylpiracetam, nefiracetam, nebracetam and coluracetam) [1, 2]. Experimental and clinical studies of racetams initially focused on their nootropic effects; later, data on their possible neuroprotective effects and use after stroke, as well as their use as antiepileptic drugs, appeared in the literature [3]. Accordingly, there is a constant growing interest in synthesising new molecules containing this pharmacophore. In this study, nebracetam (4-(aminomethyl)-1-benzylpyrrolidine-2-one) was chosen as a basic substance for chemical modification. This compound is of consistently high interest among scientists. According to the literature, it can improve linguistic learning and memory in patients with dementia [4]. Therefore, an in-depth study of methods for synthesis and the nootropic activity in several analogues of 4-(aminomethyl)-1-benzylpyrrolidine-2-one is of undoubted interest and leads to a logical conclusion about the feasibility of further determination of the relationship between the structure and action in a number of these compounds.



R = 2-Cl (**a**); 3-Cl (**b**); 4-Cl (**c**);
3-Br (**d**); 2-F (**e**); 2,4-diF (**f**);
3,5-diF (**g**); 3-Cl,4-F (**h**)

Nebracetam hydrochloride (Synonyms: WEB 1881 FU hydrochloride) is a nootropic M1-muscarinic agonist that induces a rise of intracellular Ca^{2+} concentration. Nebracetam hydrochloride exhibits an EC50 of 1.59 mM for elevating [Ca^{2+}]. Nebracetam induced a rise of [Ca^{2+}] in the medium with 1 mM Ca^{2+} and without Ca^{2+} (plus 1 mM EGTA). The nebracetam-induced [Ca^{2+}] rise was blocked by atropine greater than pirenzepine greater than AF-DX 116. From these results, nebracetam seems to act as an agonist for human M1-muscarinic receptors [5].

The search for new chemical structures that can exhibit a specific biological effect is a complex problem; its solution requires the use of modern *in silico* methods of molecular modelling. A pre-experimental study of the potential of the newly synthesized compounds was conducted using molecular docking. The aim of using the docking studies was to determine the feasibility of pharmacological studies of their nootropic activity. The *in silico* studies will allow us to assess the effect of introducing various substituents into the benzyl radical of the compounds synthesized on the manifestation of the nootropic activity and optimize the further search for new effective compounds in this series of derivatives.

One of the most important stages of docking is the selection of biological targets. Currently, there are many nootropic drugs of various chemical structures that act on the corresponding biological targets and, as a result, have a wide range of the pharmacological activity [2, 6]. At the stage of planning the research and selecting potential nootropic biotargets, the current range, chemical structure, and mechanisms of action of existing nootropic drugs were analyzed.

Phosphodiesterase inhibitors, such as Rolipram, as well as NMDA-modulating drugs, such as Modafinil and Methylphenidate, are used to enhance attention, memory, and alertness [7, 8]. Drugs based on Ginkgo biloba have neuroprotective effects; they also act as antioxidants and antiapoptotic agents and inhibit caspase-3 activation and amyloid aggregation in Alzheimer's disease [9]. Acetylcholinesterase (AchE) inhibitors eliminate the symptoms of Alzheimer's disease. However, there are only a few systematic data on the effect of treatment with acetylcholinesterase inhibitors on cognitive function in healthy individuals [10]. Taking into account the fact that most nootropic agents do not have clear mechanisms of action, there is a practical application of most of them when treating brain injuries [11].

The mechanisms of action of "nootropic racetams" have been studied less than the clinical use of these drugs, which can complicate the selection of biotargets for docking. It is known that these compounds interact with target receptors in the brain and modulate the processes of excitation or inhibition of neurotransmitters, neurohormones, and postsynaptic signals. At the neural level, piracetam modulates neurotransmission in a number of transmission systems (including cholinergic and glutamatergic), has neuroprotective and anticonvulsant properties, and improves neuroplasticity. At the vascular level, it reduces the adhesion of erythrocytes to the vascular endothelium, prevents the manifestation of vasospasm and facilitates microcirculation. Various physiological effects may be partly due to the restoration of cell membrane fluidity. A wide range of physiological effects are consistent with the use of piracetam in a number of clinical indications [12, 13]. Piracetam and Levetiracetam, an S-enantiomer, are pyrrolidone derivatives, have similar chemical structures but have different individual pharmacological profiles and, consequently, different clinical methods of use. Currently, a brain-specific stereoselective binding site, to which Levetiracetam and other S-enantiomers have a high affinity, has been identified. In preclinical studies, piracetam significantly improves learning and memory; in contrast, the effect of Levetiracetam is weaker, but it is much more active in preventing seizures in epilepsy and other seizures. Aniracetam from the racetam group activates AMPA receptors and accelerates the synaptic transmission of neural signals in the central nervous system. AMPA is one of the subgroups of glutamate receptors that are important for neural communication, memory formation, and attention. Aniracetam increases the release of norepinephrine, dopamine and serotonin, activates acetylcholine receptors and has a neuroprotective effect [14]. Pramiracetam increases the rate of sodium-dependent high-affinity choline uptake in rat hippocampal synaptosomes in vitro. It may suggest that its effect on cognitive function may occur by accelerating the flow of cholinergic neural impulses in the septo-hippocampal area [11, 15]. The affinity of Phenylpiracetam to the nicotinic acetylcholine (nACh) receptor has been demonstrated in *in vitro* ligand binding experiments. However, administering this drug to rats increases the number of both nACh and NMDA receptors but decreases serotonin and dopamine receptors in brain tissue [11]. The mechanism of action of nebracetam has been studied in experiments on rats. It was shown that nebracetam is an agonist of the muscarinic (M1) acetylcholine receptor. Later, it was confirmed that it acts as an agonist of the human M1 muscarinic receptor based on a study with human leukaemia T-cells. In laboratory animals, nebracetam had a neuroprotective effect by enhancing cholinergic and limbic noradrenergic functions of the hippocampus. According to histological data, it was found to have a protective effect on neuronal cells in the hippocampus of stroke-prone rats [16, 17].

Taking into account that the basic nebracetam and most racetams have a predominantly cholinergic mechanism of nootropic action, acetylcholine muscarinic and nicotinic receptors were chosen for docking.

The aim of research. Referring to the effective use of racetams as highly active nootropics, there is growing interest in finding new derivatives of this group of compounds. In this regard, it was decided to obtain benzyl-modified 4-(aminomethyl)-1-benzylpyrrolidin-2-ones and predict nootropic properties using molecular docking methodology.

2. Planning (methodology) of research

According to the literature data and the results of our own research, a sufficiently studied manifestation of the effect of introducing substituents into the structure of

nebracetam indicates the prospects of modifying its framework with substituents, which can additionally affect the formation of stabilization contacts with potential cognitive targets [5, 18, 19]. To optimize the search for new nootropic molecules, chemical modification of nebracetam was planned by introducing substituents into the benzyl radical (Fig. 1).

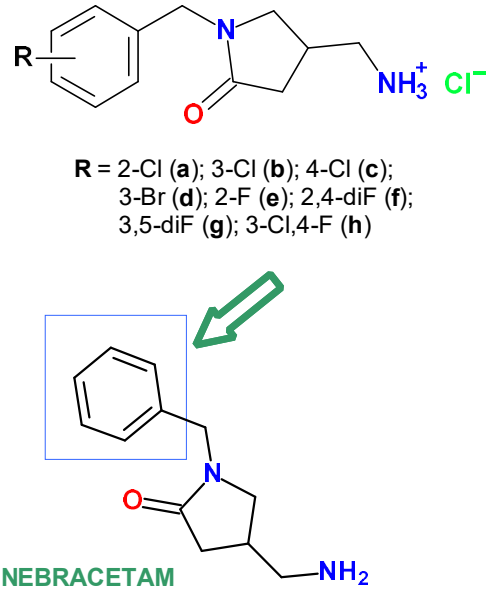


Fig. 1. A planned modification of the basic 4-(aminomethyl)-1-benzylpyrrolidine-2-one

To carry out such modification and obtain target analogues of 4-(aminomethyl)-1-benzylpyrrolidine-2-one, two methods of synthesis (basic (1) and alternative (2)) were proposed. In both methods, the introduction of substituents into the benzyl radical of 4-(aminomethyl)-1-benzylpyrrolidine-2-one was planned already at the first stage of the synthesis by using the corresponding substituted benzylamines as the starting compounds. Basic nebracetam exhibits a nootropic effect mainly through the mechanisms of choninergic neurotransmission [17]. Therefore, at the stage before docking studies, an organizer of crystallographic models was created. It was based on the analysis of the target architecture and the structural similarity of the generated objects to the reference drugs. The selected targets for docking studies are complex mechanisms with active site and multicenter allosteric modulation. In this regard, all the links that affect neurotransmission were analyzed and summarized to select the appropriate receptor domains for binding their sites with the synthesized substances. The next stage of the research is the molecular docking of optimized R and S isomers of the synthesized derivatives in order to determine the affinity to the selected targets and provide recommendations for further experimental studies and the need for further separation of the synthesized racemate mixtures [20].

3. Materials and methods

3. 1. Chemistry

Reagents manufactured by Sigma-Aldrich, USA, were used in this work. The required reagents were purified using standard techniques. Control of the reactions was carried out using thin-layer chromatography (eluent – ethyl acetate-hexane 1:2) on "Sorbfil UV-254" plates. The elemental analysis was performed on a "Hewlett Packard" automatic analyzer M-180 company. ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz spectrometers. The solvent was dimethyl sulfoxide (DMSO-d6). Chemical shifts were shown on a scale (m.ch.). LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (λmax 215 and 254 nm) using a Luna-C18 column, Phenomenex (100×4 mm). Elution started with water and ended with acetonitrile/water (95:5, v/v) using a linear gradient at a flow rate of 0.15 mL/min and an analysis cycle time of 25 min.

Yields of substances in grams are indicated in the following sequence meth1/meth2/optimized meth2:

1. 4-(Aminomethyl)-1-[(2-chlorophenyl)methyl] pyrrolidin-2-one hydrochloride (8a).

Yield in grams: 6.38/3.69/4.51, (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$-5H pyrrolidine: 2.28–2.30 (*dd*, *J*=7.2, 6.4 Hz, 1H), 2.49–2.52 (*m*, 2H), 3.06–3.10 (*m*, 2H); 2.88 (*t*, *J*=0.8 Hz, 2H, CH_2); 4.39–4.52 (*q*, *J*=15.6 Hz, 2H, CH_2); ArH: 7.30–7.34 (*m*, 3H), 7.42–7.47 (*m*, 1H); 8.07 (*s*, 3H, NH_3^+). 52.38 % C, 5.86 % H, 10.18 % N. Calculated for $C_{12}H_{16}Cl_2N_2O$. Found, %: C 52.69, H 5.94, N 10.05. LC/MC m/z: 239.0 [$(M+H)^+$].

2. 4-(Aminomethyl)-1-[(3-chlorophenyl)methyl] pyrrolidin-2-one hydrochloride (8b).

Yield in grams: 6.38/3.69/4.51; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$-5H pyrrolidine: 3.00–2.52 (*q*, *J*=3.6 Hz, 1H), 2.56–2.65 (*m*, 2H), 3.00–3.08 (*m*, 2H); 2.79 (*t*, *J*=4.8 Hz, 2H, CH_2); 4.34–4.38 (*q*, *J*=6.8 Hz, 2H, CH_2); ArH: 7.19-7.20 (*d*, *J*=6.8 Hz, 1H), 7.29 (*s*, 1H), 7.36–7.41 (*m*, 2H); 8.0 (*s*, 3H, NH_3^+). 52.38 % C, 5.86 % H, 10.18 % N. Calculated for $C_{12}H_{16}Cl_2N_2O$. Found, %: C 52.71, H 5.93, N 10.02. LC/MC m/z: 239.0 [$(M+H)^+$].

3. 4-(Aminomethyl)-1-[(4-chlorophenyl)methyl] pyrrolidin-2-one hydrochloride (8c).

Yield in grams: 6.38/3.69/4.51; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$-5H pyrrolidine: 2.28–2.30 (*dd*, *J*=7.2, 6.4 Hz, 1H), 2.49–2.52 (*m*, 2H), 3.06–3.10 (*m*, 2H); 2.79 (*t*, *J*=4.8 Hz, 2H, CH_2); 4.39–4.53 (*q*, *J*=7.2 Hz, 2H, CH_2); ArH: 7.18–7.19 (*d*, *J*=1.8 Hz, 1H), 7.22 (s, 1H), 7.36–7.39 (*m*, 2H); 8.0 (s, 3H, NH_3^+). 52.38 % C, 5.86 % H, 10.18 % N. Calculated for $C_{12}H_{16}Cl_2N_2O$. Found, %: C 52.72, H 5.93, N 10.05. LC/MC m/z: 239.2 [$(M+H)^+$].

4. 4-(Aminomethyl)-1-[(3-bromophenyl)methyl] pyrrolidin-2-one hydrochloride (8d).

Yield in grams: 7.32/4.29/5.24; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$- 5H pyrrolidine: 2.30–2.40 (*dd*, *J*=2.4, 1.6 Hz 1H), 2.49–2.54 (*m*, 1H), 2.63–2.67 (*m*, 1H), 3.068 (*m*, 1H), 3.33–3.36 (*m*, 1H); 2.86 (*t*, *J*=2.4 Hz, 2H, CH_2); 4.35–4.38 (*q*, *J*=12 Hz, 2H, CH_2); ArH: 7.22–7.24 (*d*, *J*=7.6 Hz, 1H), 7.32–7.34 (m, 1H), 7.43 (*t*, *J*=1.8 Hz, 1H), 7.47-7.49 (*dd*, *J*=1.2, 0.8 Hz, 1H); 8.19 (*s*, 3H, NH_3^+). 45.09 % C, 5.04 % H, 8.76 **%** N. Calculated for $C_{12}H_{16}BrClN_2O$. Found, %: C 45.32, H 5.27, N 8.68. LC/MC m/z: 320.2 [$(M+H)^+$].

5. 4-(Aminomethyl)-1-[(2-fluorophenyl)methyl] pyrrolidin-2-one hydrochloride (8e).

Yield in grams: 6.0/3.47/4.24; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$- 5H pyrrolidine: 2.24–2.28 (*dd*, *J*=9.2, 7.6 Hz, 1H), 2.43–2.50 (*m*, 1H), 3.06–3.10 (*m*, 1H), 3.35–3.44 (*m*, 2H); 2.86–2.87 (*m*, 2H, CH_2); 4.36–4.48 (*q*, *J*=14.8 Hz, 2H, CH_2); ArH: 7.19–7.23 (*m*, 2H); 7.26–7.28 (*m*, 1H); 7.29–7.35 (*m*, 1H); 8.26 (*s*, 3H, NH_3^+). 55.71 % C, 6.23 % H, 10.83 % N. Calculated for $C_{12}H_{16}ClFN_2O$. Found, %: C 55.96, H 6.68, N 10.97. LC/MC m/z: 223.2 [$(M+H)^+$].

6. 4-(Aminomethyl)-1-[(2,4-difluorophenyl)methyl]pyrrolidin-2-one hydrochloride (8f).

Yield in grams: 6.42/3.71/4.54; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$- 5H pyrrolidine: 2.23–2.52 (*dd*, *J*=7.6, 6.8 Hz, 1H), 2.41–2.48 (*m*, 1H), 2.624 (*m*, 1H), 3.07–3.08 (*m*, 1H), 3.34–3.35 (*m*, 1H); 2.83–2.84 (*m*, 2H, CH_2); 4.32–4.42 (*q*, *J*=15.2 Hz, 2H, CH_2); ArH: 7.06–7.09 (*m*, 1H), 7.21–7.27 (*m*, 1H), 7.32–7.34 (*q*, *J*=6.8 Hz, 1H), 8.18 (*s*, 3H, NH_3^+). 52.09 % C, 5.46 % H, 10.12 % N. Calculated for $C_{12}H_{15}ClF_2N_2O$. Found, %: C 52.35, H 5.74, N 10.57. LC/MC m/z: 241.2 [$(M+H)^+$].

7. 4-(Aminomethyl)-1-[(3,5-difluorophenyl)methyl]pyrrolidin-2-one hydrochloride (8g).

Yield in grams: 6.42/3.71/4.54; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$-5H pyrrolidine: 2.30–2.32 (*dd*, *J*=7.6, 6.8 Hz, 1H), 2.68 (*m*, 1H), 3.08–3.12 (*m*, 1H), 3.37–3.41 (*m*, 2H); 2.68–2.90 (*m*, 2H, CH_2); 4.39 (*s*, 2H, CH_2); ArH: 6.95–6.97 (*m*, 2H), 7.13–7.17 (*m*, 1H); 8.19 (s, 3H, NH_3^+). 52.09 % C, 5.46 % H, 10.12 % N. Calculated for $C_{12}H_{15}ClF_2N_2O$. Found, %: C 52.32, H 5.71, N 10.49. LC/MC m/z: 241.2 [$(M+H)^+$].

8. 4-(Aminomethyl)-1-[(3-chloro-4-fluorophenyl) methyl]pyrrolidin-2-one hydrochloride (8h).

Yield in grams: 6.63/3.93/4.80; (ethanol). ^{1}H NMR (400 MHz, DMSO-d_6) δ: C$\underline{H}_2$-C$\underline{H}$-C$\underline{H}_2$- 5H pyrrolidine: 2.24–2.52 (*dd*, *J*=7.2, 6.8 Hz, 1H), 2.41–2.48 (*m*, 1H), 2.630 (*m*, 1H), 3.07–3.08 (*m*, 1H), 3.34–3.35 (*m*, 1H); 2.84–2.87 (*m*, 2H, CH_2); 4.36–4.47 (*q*, *J*=15.4 Hz, 2H, CH_2); ArH: 7.22–7.24 (*d*, *J*=7.6 Hz, 1H), 7.32–7.34 (*m*, 1H), 7.47–7.49 (*m*, 1H); 8.19 (*s*, 3H, NH_3^+). 49.16 % C, 5.16 % H, 9.56 % N. Calculated for $C_{12}H_{15}Cl_2FN_2O$. Found, %: C 49.49, H 5.31, N 9.78. LC/MC m/z: 257.2 [$(M+H)^+$].

3. 2. Molecular docking studies

The Autodock 4.2 software package was used for receptor-oriented flexible docking. Ligands were prepared using the MGL Tools 1.5.6 program. The Ligand optimization was performed using the Avogadro program. To perform calculations in the Autodock 4.2 program, the output formats of the receptor and ligand data were converted to a special PDBQT format. The active macromolecule centre of the nootropic targets (PDB ID: 5CXV, 6PV7) from the Protein Data Bank (PDB) is used as a biological target for docking. The receptor maps were made using MGL Tools and AutoGrid programs. Water molecules, ions, and ligands were removed from the PDB file. 6PV7 A+B: coordinates *x*=140.775, *y*=168.99, *z*=159.92, size *x*=60, *y*=88, *z*=80; 5CXV A: coordinates *x*=–15.821, *y*=–18.268, *z*=59.512, size *x*=64, *y*=54, *z*=74. Visualization of the resulting complexes of the molecules studied in the active sites of the receptors was carried out using the Discovery Studio Visualizer program.

The following docking parameters were set: the translational step was 2 Å, and the torsional freedom coefficient was 0.2983. The cluster tolerance was 2 Å. The external lattice energy – is 1000, the maximum initial energy – is 0, and the maximum number of attempts – is 10 000. The number of structures in the population – 150, the maximum number of stages of energy estimation – 2500000, the maximum number of generations – 27 000, the number of structures passing to the next generation – 1, the level of gene mutation – 0.02, the level of the crossover – 0.8, the method of the crossover – arithmetic. The α-Gaussian distribution parameter was equal to 0, and the β-parameter of Gaussian distribution was 1.

Organizer of crystallographic models.

The crystallographic model of the α3β4 nicotinic receptor subtype (PDB ID: 6PV7) in complex with nicotine was chosen for docking studies [21]. The subtype was chosen because it is abundant in the habenulo-interpeduncular pathway, which modulates the mesolimbic dopamine system, the main reward pathway in the brain, leading to improved learning and behaviour [22]. The agonist/antagonist ligand binding pocket of nAChRs is located at the edge between two adjacent subunits, with one subunit being the core side and the other being the complementary side.

Ligand binding stimulates different functional states of nAChR due to conformational changes caused by the relative movement of the five subunits to each other. The architecture of the orthosteric site is well-documented [21]. An alternative to enhancing nicotinic functions in the brain is the sensitization of nAChR to activation by endogenous ligands using so-called nicotinic allosteric potentiating ligands (modulators). There is an assertion that their sites have some common elements with classical agonists but differ from them in nAChR. It has also been suggested that dipoles appearing in the site residues can be enhanced by the anionic side chain of the Asp-89 residue [23]. Binding effectors (modulators), involved in the regulation of channel opening, are also distributed at the interfaces between the extracellular and transmembrane domains. The currently known site of positive allosteric modulation of the extracellular domain is located at the interfaces of the subunits below the orthosteric site near the transmembrane domain TMD, which also contains allosteric cavities of positive/negative modulation [24].

It is well known that muscarinic receptors of the M1 subtype are present in the cerebral cortex, hippocampus, and throughout the brain and play a role in cognitive functioning [25, 26]. Therefore, a crystallographic model of the muscarinic receptor of this subtype (PDB ID: 5CXV) was chosen for molecular docking.

The acetylcholine binding site is called an orthosteric site, and it also engages the hydrophobic sites of the receptor protein and the surrounding cell membrane. It is also a binding site for both agonists and antagonists. In muscarinic receptors, allosteric modulation is represented by a large extracellular vestibule, which is a well-documented binding site for positive modulators [27]. The contraction of the ex-

tracellular vestibule may be a key feature of activation by new nootropic molecules. The transmembrane part of the receptor contains another site that allows allosteric regulation of the receptor response. It contains residues from the upper regions of the corresponding transmembrane bundles, whose primary function is to regulate the dissociation of antagonists of the orthosteric binding site by slowing their dissociation [27]. There are also three allosteric modulating sites in the cytoplasmic intracellular domain, which can play an essential role in clustering, stabilizing, and modulating receptor functions [28], in particular through interaction with the G-protein.

The subunits α and β were used for docking to nAChR, respectively, given that most of the allosteric sites of these targets are located at the edge of the subunits of each domain. It is worth noting that all domains of the selected models are conformationally mobile and may contain undocumented cavities for binding small ligands, so stepwise molecular docking was performed for virtual binding.

4. Results

4. 1. Chemistry

Methods for the synthesis of racetams are well-known and described in the literature [29, 30]. Some known methods were used to synthesize new 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one derivatives.

The synthesis of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogs (*Method 1*).

Analogues of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one by Method 1 were obtained in 6 stages according to Fig. 2.

Analogues of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one **8(a–h)** were obtained with the isolation of intermediates at each stage and the registration of the corresponding yield percentages of both intermediate and target end products.

Stage 1. A mixture of itaconic acid **1** (0.068 mol) and substituted benzylamines **2(a–h)** (0.068 mol) was heated at 130 °C for 3 hours until water was released from the reaction mixture. The mixture in the flask was cooled to room temperature. Further, the resulting products were recrystallized from ethyl acetate to obtain 1-R-benzyl-5-oxopyrrolidine-3-carboxylic acid **3(a–h)** derivatives in the form of white solids.

Stage 2. Carbonyl diimidazole (0.05 mol) was added to solutions of 1-R-benzyl-5-oxopyrrolidine-3-carboxylic acid **3(a–h)** (0.045 mol) in 50 ml of dioxane at a temperature of 80-90 °C in parts while stirring at such a rate that the rapid release of CO_2 was controlled. After the CO_2 release was completed, the mixture was stirred at 80 °C for 30 minutes. Then, the resulting solutions were cooled to 50 °C, and Na (0.009 mol) solution in 5 ml of methanol was added to them. Solutions with the resulting products were thermostated at 50 °C for 5 hours. After confirming the completeness of the reaction by TLC, water was added to the mixture, pyrrolidine-carbon ether was extracted using dichloromethane. The solvent was removed using a rotary evaporator. White crystals of 1-R-benzyl-5-oxopyrrolidine-3-carboxylic acid methyl esters **4(a–h)** were obtained.

Stage 3. Methyl esters of 1-R-benzyl-5-oxopyrrolidine-3-carboxylic acid **4(a–h)** (0.043 mol) were dissolved in methanol and cooled to 10–15 °C. Sodium borohydride (0.054 mol) was introduced into the reaction in portions for 20 minutes. At the end of adding borohydride, the mixture was stirred for 2 hours at 20–25 °C. The solvent was removed using a rotary evaporator, and the remainder was transferred to 100 ml of a saturated salt solution. The extraction was performed using ethyl acetate as an extractant; the organic phase was treated with Na_2SO_4. The solution was evaporated to give 4-hydroxymethyl-1-R-benzylpyrrolidine-2-ones **5(a–h)** in the form of white solids.

Stage 4. Anhydrous triethylamine (6.35 g, 0.063 mol) was added to solutions of 4-hydroxymethyl-1-R-benzylpyrrolidine-2-ones **5(a–h)** (0.042 mol) dissolved in 50 ml of methylene chloride CH_2Cl_2, and a mesylate chloride solution was added to 10 ml of methylene chloride (0.046 mol). The reaction was carried out by stirring for 1 hour at a temperature of 0 °C. The reaction was controlled by TLC. After the reaction was completed, CH_2Cl_2 was washed several times with a saturated salt solution. The CH_2Cl_2 layer was then dried over Na_2SO_4 and concentrated at reduced pressure to produce technical products **6(a–h)**, which were used for the next stage without further purification (83 %).

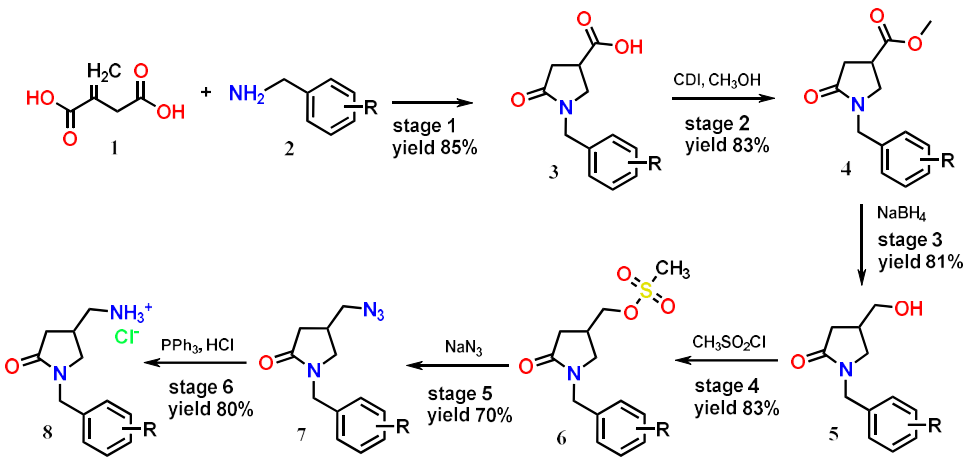


Fig. 2. The synthesis scheme *of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogs* (*Method 1*)

Stage 5. To the suspension of sodium azide (0.066 mol) in a minimum amount of DMF, 4-methanesulfonylmethyl-1-R-benzylpyrrolidine-2-one **6(a–h)** was added and heated to a temperature of 70–80 °C with stirring for 4 hours. The reaction was controlled using TLC. After completing the reaction, the solution was diluted with water, and the CH_2Cl_2 product was

extracted. Chlorine methylene was evaporated. The resulting azides **7(a–h)** were purified by chromatography on a silica gel (eluents – ethyl acetate).

Stage 6. Azides **7(a–h)** (0.029 mol) were dissolved in dry THF. Triphenylphosphine (0.034 mol) was added with a slight cooling (10–15 °C). Reactions were carried out at room temperature for 3 hours, controlling TLC. After the reaction, the solvent was evaporated, the resulting products were diluted with 5 % aqueous HCl, and the impurities were extracted with chloroform. The HCl solution was evaporated using the rotor, and pure 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one hydrochlorides **8(a–h)** were obtained.

Taking into account the need to use sodium azide, which is a highly toxic and hazardous substance, in the basic method of obtaining 4-(aminomethyl)-1-R-benzylpyrrolidine-2-ones, an alternative synthesis method has been proposed. The advantages of this method are to reduce the number of stages from 6 to 4 and avoid the need to use sodium azide.

An alternative synthesis of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogs (*method 2*).

To reduce the number of synthetic stages and increase the yield of target products, an alternative method of synthesis was proposed.

Analogues of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one by Method 2 were obtained in 4 stages.

Stage 1. The first stage of synthesis was aimed at obtaining 1-R-benzyl-5-oxopyrrolidine-3-carboxylic acid **3(a–h)** by interacting solutions of itaconic acid **1** and substituted benzylamines **2(a–h)** in toluene by boiling for 5–6 hours with a Dean-Stark nozzle until the equimolar amount of water was removed. The mixtures were left to harden while stirring for 4 hours. The precipitations were filtered out, washed with a small amount of toluene and dried in the air.

Stage 2. To a 30 % solution of acids **3(a–h)** in dioxane, portions of CDI (1.1 eq.) were added. The reaction mixtures were mixed for 3 hours at room temperature, and ammonia water (10 eq. calculated with reference to pure ammonia) in the form of a 50 % solution in dioxane was added in one step. The mixtures were stirred for 5 hours, dioxane was distilled on a rotary evaporator, the residues were diluted with water and acidified with a 0.1 M solution of hydrochloric acid to pH=5-6. The precipitates were filtered and dried in the air, obtaining the corresponding amides **9(a–h)** in the form of beige crystals.

Stage 3. Thionyl chloride (2.5 eq.) was added to the suspension of amides in pyridine (6 eq.) when cooling. The mass obtained was mixed for 12 hours, poured into ice, methylene chloride was added, and the resulting mixture was passed through celite. The organic layer was separated, while the water layer was extracted with chlorinated methylene. The combined organic extracts were washed with a 0.1 M solution of hydrogen chloride, then dried over sodium sulfate, concentrated and applied to a silica gel column for flash chromatography (eluents – ethyl acetate: hexane 6:4). Fractions containing the target substance were combined and evaporated to obtain target products **10(a–h)**.

Stage 4. Ammonia water (5 eq. calculated with reference to pure ammonia) and Raney nickel (5–7 % by weight) were added to 30 % nitrile solutions **10(a–h)** in methanol. The resulting mixture was hydrogenated under hydrogen pressure (1.1–1.3 atm) and with intensive mixing for 10–12 hours. At the end of the process, the mixture was filtered through celite and concentrated. The residues obtained were evaporated several times with toluene, obtaining 4-(aminomethyl)-1-R-benzylpyrrolidine-2-ones **8(a–h)** in the form of a brown oil. The product was diluted with 5 % aqueous HCl, the impurities were extracted with chloroform, then the HCl solution was evaporated using the rotor, and pure hydrochlorides of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogues were obtained.

The synthesis scheme of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogues (Method *2*) according to Fig. 3.

To reduce the time and number of synthetic stages, the alternative method (method 2) of the synthesis, which we consider a priority compared to the basic method (method 1), was optimized. It was experimentally determined that an effective approach to increase the yields of target compounds is to increase the heating of the reaction mixtures for up to 10 hours in the first stages of synthesis (yield 93 %) and conduct hydrogenation of the reaction mixture obtained at the third stage of Method 2, under a hydrogen pressure of 1.5 atmospheres (yield 86 %).

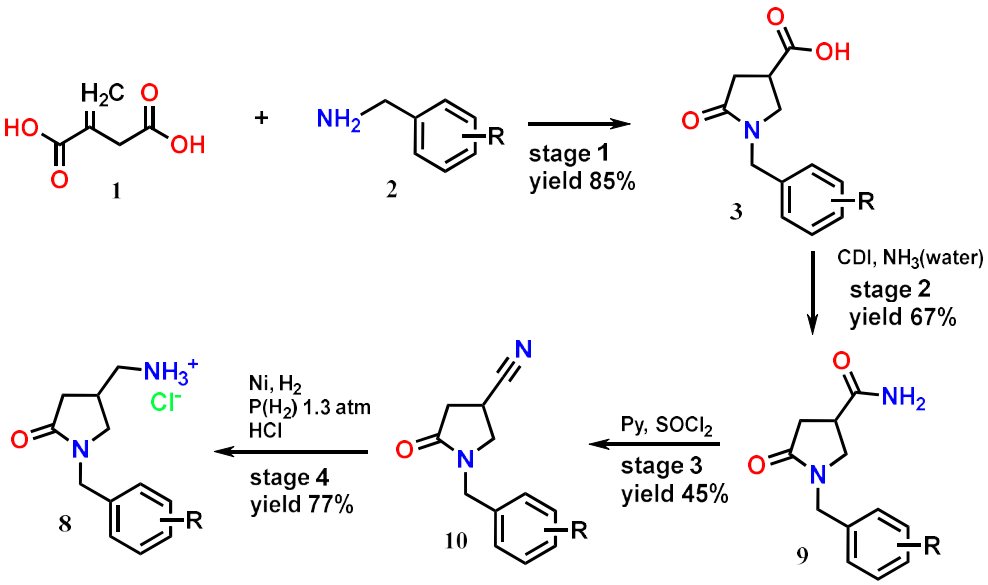
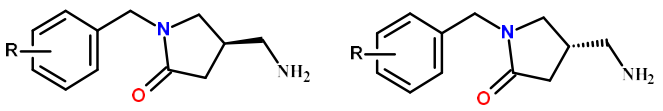


Fig. 3. The synthesis scheme of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one analogs (method 2)

The structures of the substances **8(a–h)** obtained by the optimized alternative method 2 were confirmed by the elemental analysis, ^{1}H NMR and chromatography-mass spectroscopy.

4. 2. Molecular docking

The synthesized substances and the reference drug nebracetam contain an asymmetric carbon atom, so they can exist as two enantiomers, R and S (Fig. 4). The synthesized substances were subjected to molecular docking taking into account isomerism.



R-isomer **S-isomer**

R = 2-Cl (8a); 3-Cl (8b); 4-Cl (8c); 3-Br (8d); 2-F (8e); 2,4-diF (8f); 3,5-diF (8g); 3-Cl,4-F (8h)

Fig. 4. R and S structures of synthesized 4-(aminomethyl)-1-bezylpyrrolidin-2-one derivatives

Since there are no crystallographic models of known racetam nootropics co-crystallized with the selected peptides, and their activity has been established based on pharmacological studies, the molecular docking procedure was also performed for reference drugs (Piracetam, Nebracetam, Pramiracetam), and the scoring functions obtained for them were used as standard ones.

Docking studies of the synthesized compounds into the nicotinic acetylcholine receptor show that all tested molecules had no agonistic/antagonistic effect on the orthosteric site but were bound to centres other than the main site. Given that the allosteric sites of this target do not have a clear evidence base regarding the location, it might only be assumed that most of the tested molecules can act as potential ligands of allosteric modulation (effectors).

The group of new substances and the reference drugs Nebracetam and Pramiracetam docked with the target at known sites of positive allosteric modulation of the extracellular domain at the subunit interfaces, which is located below the orthosteric site near the transmembrane domain (site 1) and at a possible allosteric site located above the orthosteric site between the two subunits (site 2). It should be noted that the docking location depended on the enantiomeric conformation of the tested molecules. Table 1 shows the calculated scoring functions of the R and S isomers at allosteric sites relative to the studied peptide. The remaining enantiomers and piracetam bound in non-complex cavities of different interfaces, so despite having efficient docking values (Table 1), they are not likely to have a significant allosteric effect.

As a result of the analysis of the geometric arrangement of molecules at site 2 and comparison of the calculated estimated values, molecule 8f (S-isomer) was found to have the best energy position (Affinity *dG*=–6.5 kcal/mol) with favourable intermolecular interactions for the formation of a stable complex with the target. In turn, the same enantiomeric conformation of nebracetam had value at the level of this compound. The formation of a stable complex of molecules at the site is mainly due to hydrogen bonds between the amide fragment, the oxygen atom of pyrrolidine-2-one, and the residues of Ile18 and Trp86, respectively. Fig. 5 visualizes this interaction. The study of the molecules' geometric location at the site showed the presence of the characteristic aspartic acid residue Asp89, which plays the role of dipole amplifier at the binding site of classical allosteric nicotinic target potentiators. The tested compounds interact with the Asp89 residue mainly through Van der Waals forces (Fig. 5), but other bonds are also observed; for example, the R-isomer of compound 8f (Affinity *dG*=–6.1 kcal/mol) forms a halogen bond through the Fluorine atom at the phenyl fragment (Fig. 6). Such an uncharacteristic interaction is because the R-conformation has an energetically favourable position at the site with different binding potential, although the affinity was almost at the level of S-Nebracetam.

The results of the molecular docking of the synthesized substances to the muscarinic acetylcholine receptor indicate that among the tested molecules, the substances with the best affinity values as agonists and allosteric modulators were found. Their location of the energetically favourable positions depended on the enantiomers' conformations (Table 2).

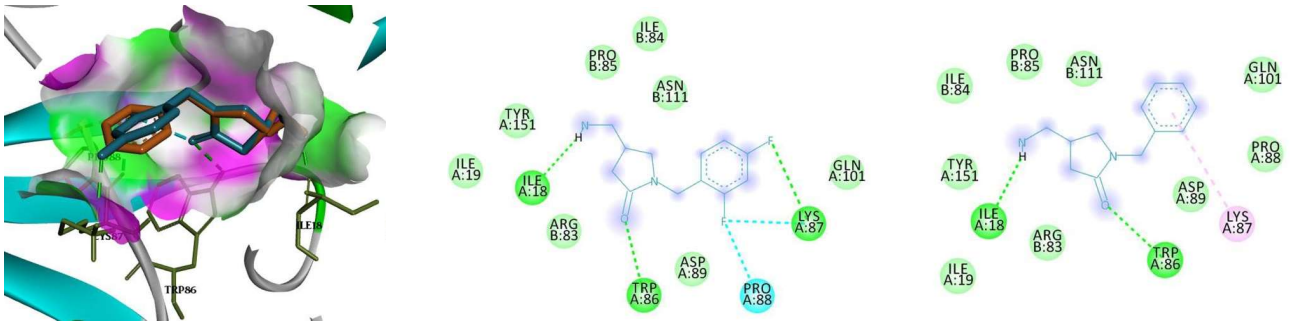
The estimated docking values indicate that the best positions were observed in the agonistic (orthosteric) site; in particular, molecule 8b (R and S) had absolute values of the scoring function at the level of the reference drug nebracetam and exceeded these values compared to piracetam. The analysis of the geometric arrangement of the R-isomer of molecule 8b and R-Nebracetam revealed that the formation of the complex is facilitated by hydrogen bonds with the amino group and the carbonyl oxygen of pyrrolidine and residues Thr189 and Ala196, Asn382, respectively, and the characteristic hydrophobic interactions (π-π π-σ, π-Alk) with amino acids Tyr404, Tyr381, which are typical for the binding of muscarinic receptor agonists. π-Sulfur and π-Alk interactions with the cysteine residue Cys407 facilitate additional stabilization of the complexes (Fig. 7).

Table 1

Estimated docking values (Affinity DG) of the studied derivatives at allosteric sites and unknown/inactive cavities of the nicotinic acetylcholine receptor (nAChR)

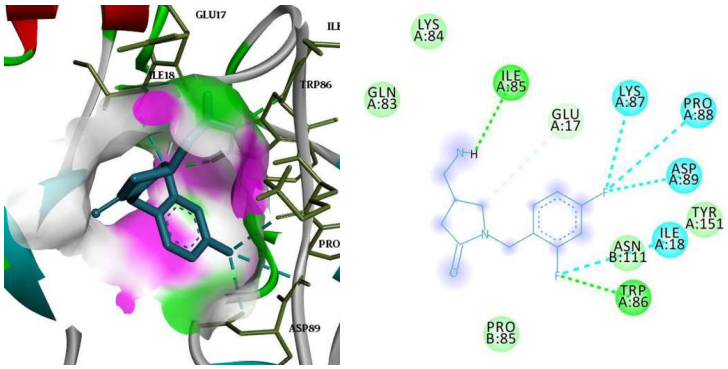
Molecule	Affinity DG, kcal/mol	Molecule	Affinity DG, kcal/mol
Site 1		Site 2	
R-8a	–6.1	S-8a	–6.0
R-8b	–6.2	S-8e	–5.8
R-8d	–6.3	R-8f	–6.1
S-8d	–5.7	S-8f	–6.5
R-8h	–6.2	S-8g	–6.3
R-Nebracetam	–5.9	S-Nebracetam	–6.3
		Pramiracetam	–5.4
unknown/inactive cavities			
S-8b*	–5.7	R-8e**	–5.6
R-8c*	–6.2	R-8g*	–5.9
S-8c*	–5.9	S-8h*	–5.7
		Piracetam	–5.1

Notes: * *– β-subunit of the transmembrane domain (TMD);* ** *– β-subunit of the agonist-binding domain* (*ABD*).



a *b* *c*

Fig. 5. Superposition of molecule 8f (S-isomer) (blue) and S-Nebracetam (orange) at the allosteric site of the agonist-binding domain (ABD) of the nAChR receptor (*a*); diagrams of intermolecular interactions of molecule 8f (S-isomer) (*b*); S-Nebracetam (*c*)



a *b*

Fig. 6. Superposition of molecule 8f (R-isomer) at the allosteric site of the agonist-binding domain (ABD) of the nAChR receptor (*a*); diagram of intermolecular interactions (*b*)

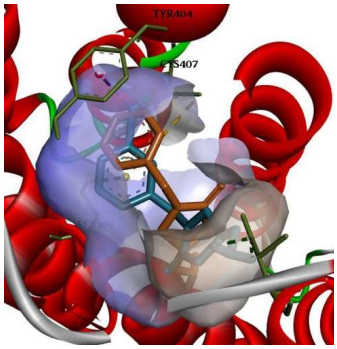
Table 2

Estimated docking values (Affinity DG) of the studied derivatives to the muscarinic acetylcholine receptor (mAChR)

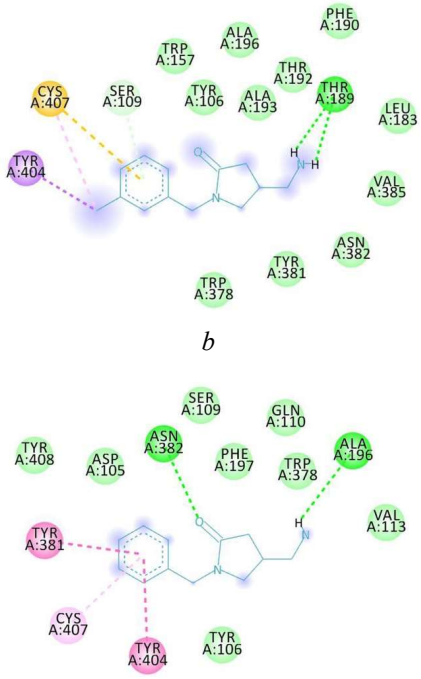
Molecule	Affinity DG, kcal/mol	Molecule	Affinity DG, kcal/mol
orthosteric site		allosteric site 1*	
R-8b	–7.9	R-8a	–6.5
S-8b	–7.4	R-8c	–6.2
S-8d	–7.9	S-8c	–6.1
R-8e	–8.1	R-8d	–6.4
S-8h	–7.7	S-8e	–6.2
R-Nebracetam	–7.7	R-8f	–6.7
S-Nebracetam	–7.6	S-8f	–6.6
Piracetam	–5.2	S-8g	–6.9
		R-8h	–6.8
allosteric site 2**			
R-8g	–6.4	S-8a	–6.4
		Pramiracetam	–6.2

*Note: * – allosteric site of extracellular domain (ECD) – extracellular vestibule; ** – allosteric site of transmembrane domain (TMD).*

The formation of the S-isomer complex with the receptor was facilitated by hydrogen bonds between the amino groups and the Thr189 residue and the corresponding hydrophobic and donor-acceptor interactions (**π-π π-σ,** **π**-Alk, π-H, π-Sulfur) of the substituted and unsubstituted phenyl fragment with Tyr404, Tyr381, Trp378, and Cys407 (Fig. 8).

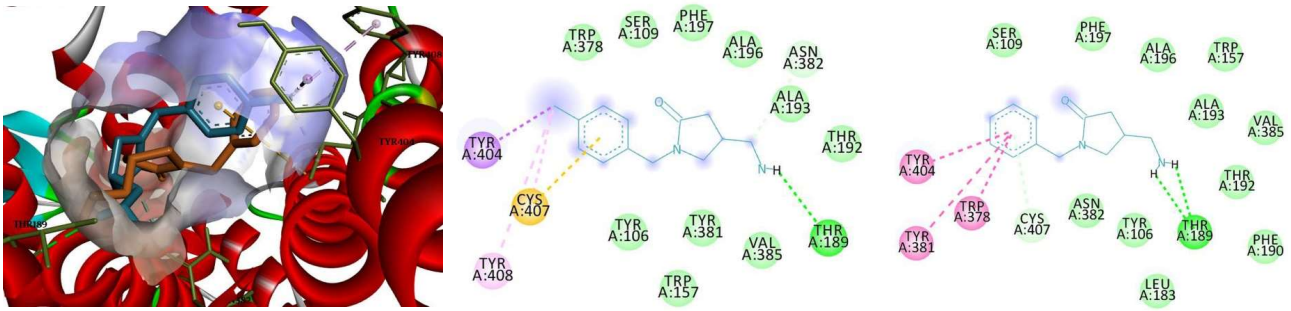


a



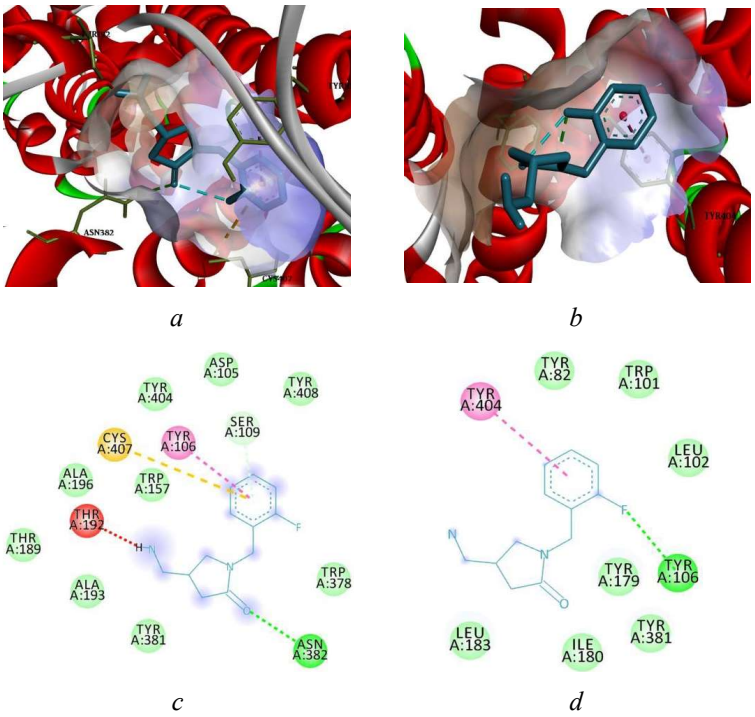
b *c*

Fig. 7. Superposition of molecule 8b (R-isomer) (blue) and R-Nebracetam (orange) at the orthosteric site of the mAChR receptor (*a*); diagrams of intermolecular interactions of molecule 8b (R-isomer) (*b*); R-Nebracetam (*c*)



a *b* *c*

Fig. 8. Superposition of molecule 8b (S-isomer) (blue) and S-Nebracetam (orange) at the orthosteric site of the mAChR receptor (*a*); diagrams of intermolecular interactions of molecule 8b (S-isomer) (*b*);S-Nebracetam (*c*)



a *b* *c* *d*

Fig. 9. Superposition of molecule 8e (R-isomer) at the orthosteric site (*a*) and molecule 8e (S-isomer) at the extracellular vestibule (*b*) of the mAChR receptor; diagrams of intermolecular interactions of molecule 8e (R-isomer) (*c*) and molecule 8e (S-isomer) (*d*)

5. Discussion

The results of our previous studies have shown that the 1-benzyl-pyrrolidin-2-one scaffold is successful in the creation of multipurpose molecular hybrids capable of reducing cognitive dysfunction [18]. Therefore, the proposed modification by introducing halogen substituents into the benzyl radical may affect the formation of stabilizing contacts with potential cognitive targets, and the established effective substitution may be the basis for the creation of new "synthetic matrices" for the search for innovative nootropics. To implement this strategy and obtain the target analogues of 4-(aminomethyl)-1-R-benzylpyrrolidin-2-one, it was proposed to adopt the classical method for the preparation of nebracetam described in the literature [30] (method 1) to obtain its benzyl-substituted derivatives. An alternative, more environmentally friendly method 2, which has the advantages of reducing the duration and number of synthesis steps and avoiding the use of sodium azide, a highly toxic and hazardous substance, was also developed. In this work, it has been proven for the first time that both methods can be used for the synthesis of structurally similar analogues. The conclusion about the formation of target products and the possibility of isolating them in their pure form has been made on the basis of the analysis of NMR and chromatography-mass spectra.

It should also be noted that molecule 8e (R-isomer) had a higher affinity (Affinity *dG*=–8.1 kcal/mol) than the reference drugs. Its opposite enantiomer had an energetically favourable position in the extracellular vestibule within the tangential regions to the orthosteric centre, namely the network of amino acid residues that promotes energy communication between the centres and mediates conformational changes in the extracellular vestibule, leading to its contraction (Fig. 9). Visualization of the interactions of the R-isomer at the orthosteric site revealed that the formation of the complex involves an unfavourable donor-donor interaction between the amino group and the Thr192 residue (Fig. 9). This may adversely affect the activation of the receptor response, given that the amine fragment of this class of compounds is involved in the formation of strong hydrogen bond with the corresponding amino acid residues of the site, which contribute to the conformational changes of the receptor.

The processed array of calculated digital docking values and a detailed analysis of the geometric arrangement of the synthesized compounds at well-documented acetylcholine receptor sites indicate that all tested molecules will contribute to the manifestation of nootropic activity to varying degrees through the mechanisms of cholinergic neurotransmission. As expected, the synthesized compounds showed the best affinity for the muscarinic target, which is consistent with pharmacological studies of the basic nebracetam [5]. Depending on the enantiomeric configuration, the studied derivatives formed complexes with the target mainly through the orthosteric and allosteric sites (extracellular vestibule), where they had extensive interactions with the corresponding amino acid residues (Tyr404, Tyr106, Tyr381,

Trp378, Cys407, Thr192, Ala196 i Asn382). The virtual screening revealed molecules with values equal to or higher than the absolute values for nebracetam and higher than the absolute values for Piracetam and Pramiracetam. It indicates the prospects of modifying the "nebracetam scaffold" at the benzyl fragment with halogen substituents. According to the docking results, the effect of isomerism does not significantly affect the affinity for cholinergic targets, so there is no need to separate the racemate mixture for further experimental screening.

Practical Relevance. The proposed studies of 4-(aminomethyl)-1-R-benzylpyrrolidin-2-one derivatives can be used for the synthesis of a wider range of substances of this series and to optimize the molecular docking procedure according to the chosen direction.

Study limitations. The proposed synthesis methods are multistep, so the yields of the target products are not high enough, and the use of toxic reagents (method 1) is not consistent with green chemistry approaches. The use of molecular docking methodology for activity prediction is advisory, so pharmacological screening should be performed to confirm the docking results.

Prospects for further research. The developed methods for the synthesis of substituted 4-(aminomethyl)-1-R-benzylpyrrolidin-2-ones can be used to design new potential nootropics. For instance, the formed intermediate azides (7a–h) are interesting and useful reagents for click reactions with the formation of the corresponding 1,2,3-triazoles. As a result, the additional appearance of this pharmacophore in the molecule may contribute to the appearance of activity, potentiation of the desired nootropic effect, and reduction of toxicity. The results of docking studies have shown the prospects of the obtained derivatives as nootropic agents; therefore, they can be recommended for experimental screening.

6. Conclusion

Based on the experimental synthetic studies, two effective methods (1 and 2) for synthesizing new analogues of 4-(aminomethyl)-1-R-benzylpyrrolidine-2-one have been proposed. An optimized alternative method (method 2) has been chosen as a priority for the synthesis of Nebracetam analogues. The results of molecular docking showed that the obtained compounds contribute to the manifestation of nootropic activity to varying degrees through the mechanisms of cholinergic neurotransmission, as evidenced by the calculated docking values in relation to the muscarinic target. It was also found that, depending on the enantiomeric configuration, the molecules formed stable complexes with the target and had characteristic binding modes both in the orthosteric site and in the extracellular vestibule (the site of positive allosteric modulation of mAChR).

Conflicts of interest

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

Data will be provided upon reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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