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Bioactive Compounds in Health and Disease

Synthesis and neurotropic activity of new derivatives of some amino acid hydantoins and their lithium salts

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ABSTRACT

Background: Amino acid hydantoins are widely used in various fields, particularly in pharmacy. For example, phenytoin is used to treat generalized epileptic seizures.

Objective: This study aims to investigate the neurotropic properties of new amino acid hydantoin derivatives in order to identify new anticonvulsants with psychotropic properties.

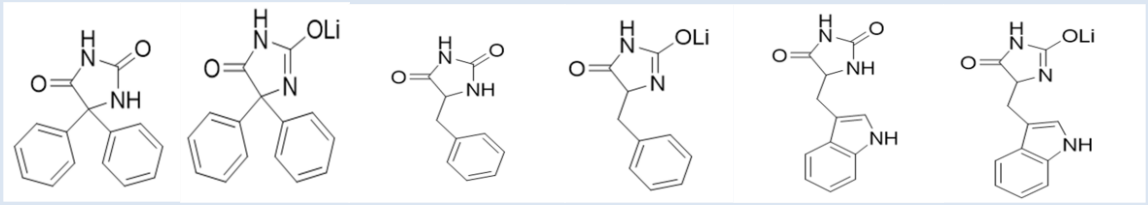
Methods: The compounds mentioned exhibited anticonvulsant properties that were evaluated through a series of tests: maximal electric shock, pentylenetetrazole, thiosemicarbazide, picrotoxin, strychnine, nicotine, and camphor convulsions on outbred mice. The psychotropic effects of the compounds were assessed through various tests, including the elevated plus maze (EPM), forced swimming, and open field tests. Additionally, their effect on the activity of monoamine oxidase (MAO) was investigated under *in vitro* conditions. The neurotoxic effect of these compounds was further examined by conducting the ''rotating rod'' test on mice.

Results: The five studied compounds, which are byproducts of amino acid hydantoins, along with Phenytoin and their lithium salts, display neurotropic properties, demonstrating anticonvulsant and psychotropic effects. Compounds that inhibit clonic pentylenetetrazole, maximal electroshock generalized tonic convulsions, nicotine, and kamphora convulsions, as well as exhibiting antithiosemicarbazide action in animals, display pronounced anxiolytic and behavior-activating effects across various internationally recognized models. Simultaneously, the compounds show antidepressant (evidenced by the "forced swimming" model) and anti-MAO effects. The compounds did not demonstrate muscle relaxant effects in the doses examined. In certain aspects of their neurotropic properties, the compounds outperformed drugs currently used in the clinic, including Phenytoin, Ethosuximide, and lithium chloride (antimanic drug), among others.

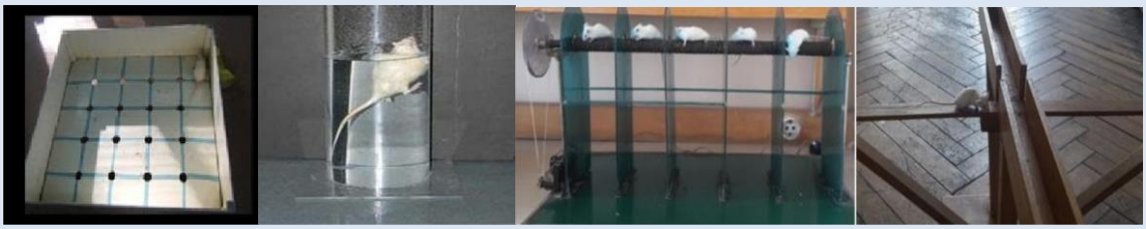
Conclusion: Hydantoins derived from DL-tryptophan, DL-β-phenyl-α-alanine, and Phenytoin, along with their corresponding lithium salts, were synthesized. Both the anticonvulsant and psychotropic effects of these substances have been thoroughly studied. In several models, compounds that inhibit maximal electroshock convulsions in animals and clonic pentylenetetrazole also display anxiolytic and behavior-activating effects. These compounds also exhibit antidepressant and anti-MAO effects. The investigated compounds can be used in medicine, as drugs, in the treatment of epilepsy with psychotropic disorders.

Keywords: antiepileptic drugs, derivatives of amino acid, lithium salts, neurotropic activity, pentylenetetrazole convulsions.

Graphical Abstract: Phenytoin and Li-salt, Hydantoin of *D,L*-Phe and Li-salt , Hydantoin of *D,L*-Trp and Li-salt (1,4,2,5,3,6)

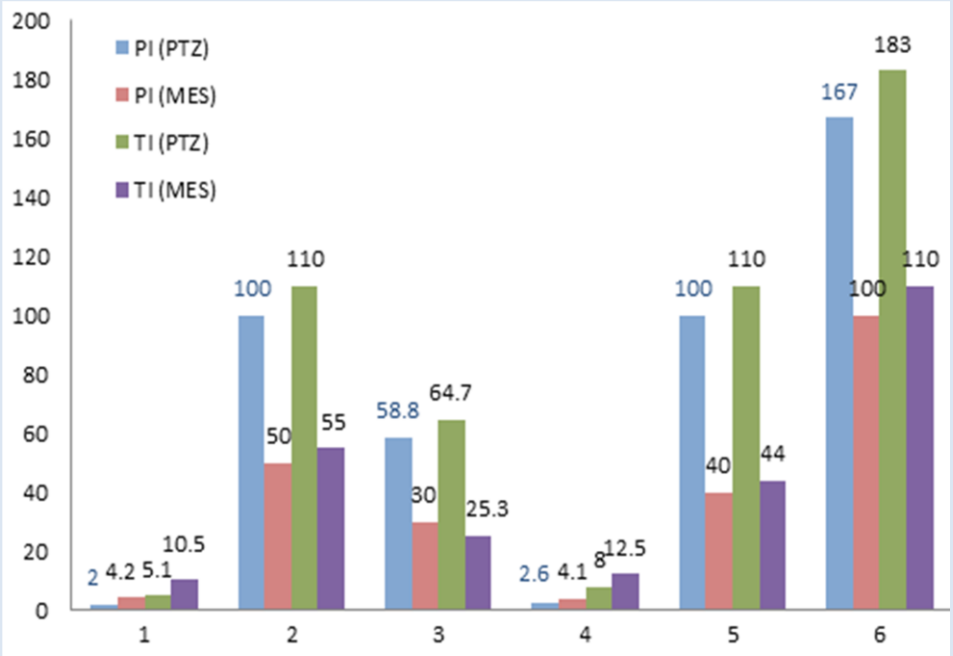


1 **4** **2** **5** **3** **6**



«open field» **«porsolt»** **«rotating rod»** **«EPM»**

Safety of new synthesized compounds (1-6) via protective (PI) and therapeutic (TI) indexes by pentylenetetrazole (PTZ) and maximal electroshock (MES) seizure



INTRODUCTION

Epilepsy is the most prevalent neurological disorder, and its frequency in developed countries ranges from 0.5% to 1% [1-2]. It is defined by recurrent and sudden unprovoked seizures, where bursts of neuronal hyperactivity occur in the brain. If confined to a specific area of origin, the seizures can manifest as "focal" or "partial", but if they spread throughout both cerebral hemispheres, they are considered "generalized." Convulsions emerge due to an imbalance between excitatory and inhibitory systems within the central nervous system. Based on distinct electrophysiological and clinical characteristics, seizures have recently been categorized into focal and generalized convulsive, as well as non-convulsive epilepsies [3-4].

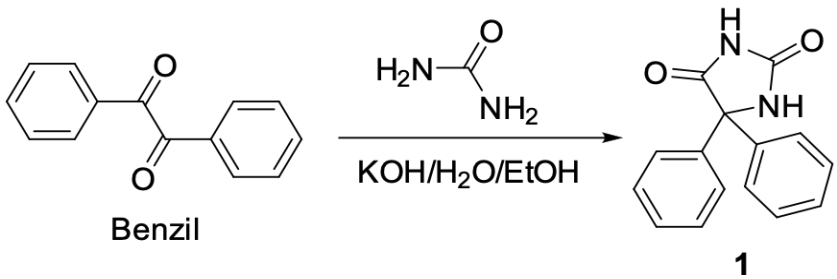
Pharmacotherapy is the mainstay of treatment for epilepsy and symptomatic seizures. The duration and continuity of drug therapy, individual selection of the drug for each patient, the high frequency of adverse reactions, as well as the prevalence of pharmacoresistant forms of the disease make epilepsy one of the most pressing problems of modern medicine [5-6]. Therefore, the issue of developing new anticonvulsants that affect various mechanisms of the occurrence and development of convulsive conditions is relevant [7-8]. The growth of psychoneurological diseases with an increasing proportion of borderline mental disorders, accompanied by cognitive deficits, anxiety-depressive symptoms, asthenoneurotic manifestations, and a decrease in physical and mental performance, makes it urgent to search for and develop new highly effective and low-toxic neuropsychotropic drugs with a broad spectrum of activity, thus allowing pharmacological intervention for a range of psychoneurological symptoms and diseases. It is important to consider the criteria for modern drugs used in neurological practice, like the effectiveness and breadth of the pharmacological spectrum (possibility and feasibility of use in various forms of pathology) and safety regulations, and also for a healthy diet [9-11].

Essentially, anticonvulsant drugs include derivatives of compounds such as hydantoin, barbituric acid, benzodiazepine, and others. Phenytoin, more commonly known as Difenin or Dilantin, is among the widely recognized anticonvulsants of the hydantoin derivatives class [12-15]. Amino acid hydantoin derivatives are essential to several fields, such as cosmetology and the pharmaceutical industry. Their scope ranges from antiseptic additives in cosmetics to the relief of manic syndrome in manic-depressive psychosis.

Hydantoins of DL-tryptophan and DL-β-phenyl-α-alanine and Phenytoin, as well as their respective lithium salts, were synthesized. Thorough investigations have been conducted on the psychotropic and anticonvulsant properties of these substances. The work is a continuation of previously conducted studies [16-18].

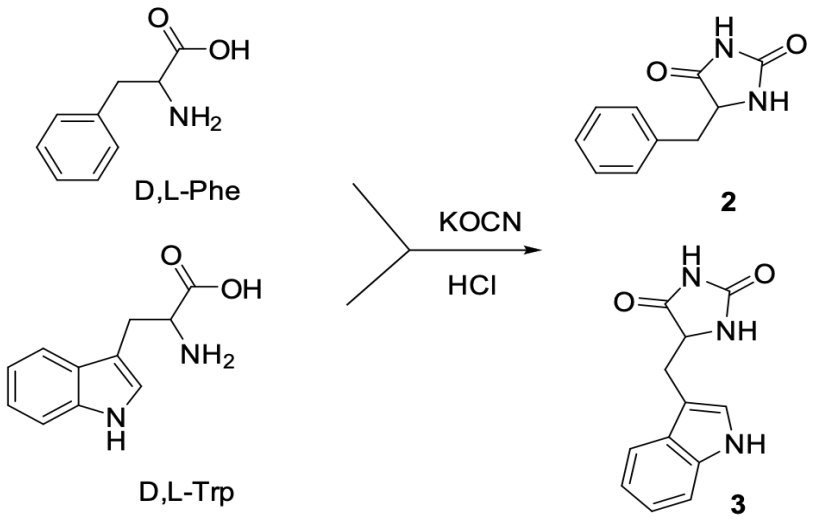
Phenytoin (**1)** was obtained by the reaction of benzyl and urea in an aqueous-alcoholic KOH solution according to scheme 1.

Scheme 1:



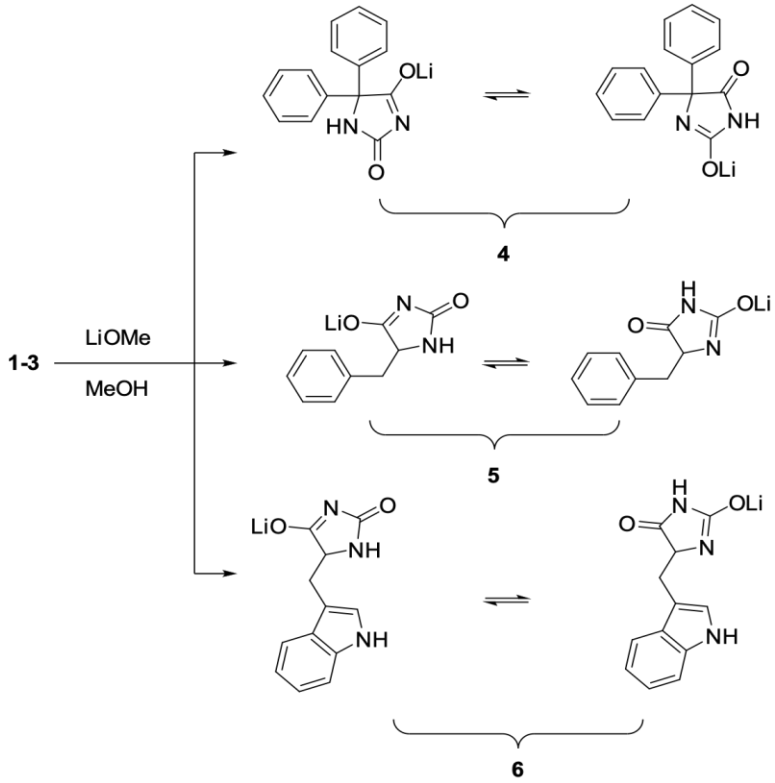
(*D,L*)-5-Benzylimidazoline-2,4-dione (**2**) and 5-((1*H*-indol-3-yl)methyl)imidazolidine-2,4-dione **(3**) were obtained, respectively, from *D,L*-β-phenyl-α-alanine and *D,L*-tryptophan by reaction with potassium cyanate in the presence of HCl, according to scheme 2.

Scheme 2:



Lithium salts of hydantoins 4–6 were obtained by reacting products 1–3 with lithium methoxide in methyl alcohol according to Scheme 3.

Scheme 3:



[19-21].

PHARMACOLOGICAL RESEARCH METHODS

All procedures were conducted in compliance with the EU Directive on the protection of animals used for scientific purposes - EU Directive 2010/63/EU, adopted on September 22, 2010.

Evaluation of the Anti-convulsant Activity of the Synthesized Compounds: The anticonvulsant spectrum of action of the compounds was studied on mice of both sexes, weighing 18-24 g, using the following tests: pentylenetetrazole (PTZ), maximal electric shock (MES), thiosemicarbazide (TSC), picrotoxin, strychnine, nicotine, camphor convulsions, and arecoline tremor [22-25]. The PTZ test serves as an experimental model for eliciting absence and myoclonic seizures and anticipating compounds'

anxiolytic properties. In the PTZ test, mice were administered an 80 mg/kg dose of analeptic subcutaneously, and the efficacy of the drugs was assessed based on their ability to prevent clonic seizures.

The compounds' anticonvulsant activity was also evaluated by preventing the tonic-extensor phase of a convulsive seizure of maximal electric shock (MES). The parameters of the maximal electric shock are 50 mA, duration—0.2 sec, and oscillation frequency—50 impulses/sec. The evaluation criterion includes the prevention of the tonic-extensor phase of a convulsive seizure.

Picrotoxin is a blocker of GABA inhibition [26], and thiosemocarbazide (TSC) is an inhibitor of glutamic acid decarboxylase, an enzyme responsible for the synthesis of gamma-aminobutyric acid (GABA) [27]. In our studies, picrotoxin was administered subcutaneously as a 0.1% solution at a dose of 5-6 mg/kg of animal weight. The latent period of picrotoxin seizures in control and experimental animals, the presence of seizures, their intensity, and survival rate after 24 hours were determined. TSC was administered at a dose of 18 mg/kg subcutaneously. In the experiments, the latent period, the incidence of seizures, and mortality after 24 hours were recorded [28]. Strychnine inhibits GABA receptor binding and is a standard model of tonic seizures [29]. Strychnine nitrate is injected subcutaneously into the cervical back region as a 0.2% solution at a dose of 1.5-2 mg/kg of animal weight. Animals are observed for 30-60 minutes after the injection of strychnine. The results were considered in an alternative form, and the average latent period for the appearance of strychnine seizures before and after administration of the studied compounds was also calculated (in minutes). Arecoline tremor and nicotine convulsions in mice are widely used to identify possible M- or N-anticholinergic activity in drugs. The nicotine test in our studies was carried out by administering nicotine to experimental animals (mice) at a dose of 8 mg/kg. To obtain arecoline hyperkinesis and tremor in our studies, we administered arecoline subcutaneously after pre-administered drugs at a dose of 15 mg/kg. The criterion for assessing the effect was the absence of convulsions or weak convulsions, affecting only the forelimbs and expressed in small tremors in the limbs and head [22].

The test for camphor cramps is included in the methodological recommendation for conducting preclinical studies of drugs, ed. Mironov A.N. (2012) [28]. Our experiments revealed camphor convulsions after the intraperitoneal injection of 0.04 ml of 5% slightly warmed camphor oil in white mice of 20 g (1 g/kg animal weight). The effect of drugs on survival and the nature of seizures caused by camphor are being studied. The experimental results were considered in an alternative form, and the active compounds' average effective doses were determined.

The substances were administered intraperitoneally at doses ranging from 10 to 300 mg/kg, in suspension with carboxymethylcellulose (“Viadi – Ingredients”) and Tween-80 (“Ferak Berlin”). Administration occurred 45 min before the administration of convulsants and the application of electrical stimulation. Control animals were given an emulsifier. Each dose of compound in each test was studied in 5 animals. The comparison analogs were the anticonvulsant drug Phenytoin (5, 5-diphenylhydantoin - compound №. 1), which is a structural analog of the studied compounds, and the antimanic drug, lithium chloride [12, 22]. The acute daily toxicity of the drugs was tested in mice. The experimental results were statistically processed by calculating their effective 50% (ED_{50}), lethal 50% (LD_{50}), and neurotoxic 50% (TD_{50}) doses [30] as well as the use of Student's t-test. Protective ($TI=TD_{50}/ED_{50}$) and therapeutic ($TI=LD_{50}/ED_{50}$) indices were calculated.

Evaluation of the Psychotropic Properties of the Synthesized Compounds: The compounds' psychotropic properties and effect on monoamine oxidase were investigated using the following tests: "elevated plus maze-EPM," "open field," and "forced swimming." The exploratory-motor behavior of rats

weighing 120-140 g. was studied using a modification of the ''open field'' model [31-32]. Therefore, an installation featuring a bottom divided into squares with holes (cells) was utilized. The experiments were carried out in the daytime under natural light. During the 5-minute experiment, we asssessed indicators of activating and sedative behavior, including standing on the hind legs (vertical movements), the number of horizontal movements, and sniffing the cells. Each compound, control, and comparison analogs, in this model, had 8 animals. The most effective dose for administering the test compound to rats was determined to be 50 mg/kg, given intraperitoneally.

Anti-anxiety, antidepressant, and sedative effects were studied using the “elevated plus maze” model in mice, developed by S. Pellow and co-authors (1986) [33]. Typically, normal animals display a preference for spending the majority of their time in the closed arms of the maze. The drug’s anxiolytic effect is evaluated by an increase in the number of entries into open sleeves and the time spent in them without an increase in general motor activity. The duration spent in the closed arms and the number of attempts to enter the center of the installation are also recorded. In the above model, test compounds and comparison drugs were administered intraperitoneally prior to the experiments. Control animals were administered an emulsifier. The results were processed statistically ($P \leq 0.05$).

The “forced swimming” model was used to assess “despair and depression” [34-36]. Experimental animals were required to swim in a glass container (height 22 cm, diameter 14 cm) filled 1/3 of the way with water. Initially, intact mice displayed vigorous swimming activity, but eventually, they were forced to immobilize. The latency period of immobilization, as well as the total duration of active swimming immobilization, was fixed for 6 minutes. Experiments were conducted under natural lighting conditions.

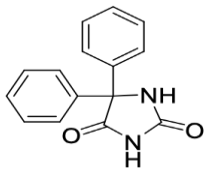
5,5-Diphenylimidazolidine-2,4-dione (Phenytoin) (1):

Investigation of antimonoaminoxydase effects: A study of the effect of amino acid hydantoins on monoamine oxidase (MAO) activity in bovine brain tissue and liver was carried out in vitro. The monoamine oxidase (MAO) source was a 50 % bovine brain homogenate. This was achieved by homogenizing the brain in a glass homogenizer with an equal weight volume of 2.5 % Arcopal solution [37]. The substrate used in this experiment was Serotonin (5-OT) creatinine sulfate monohydrate, which was added to the samples following a 30-minute preincubation of the enzyme with the test substance at room temperature. Each compound underwent testing in 4 separate experiments, from which the average data were derived. The control drug used was indopane.

Evaluation of Incoordination of Movements in the Rotating Rod Test: The potential neurotoxic effects of the substances have also been studied. Myorelaxation was assessed using the “rotating rod” test in mice [21, 38]. In this test, mice were positioned on a metal rod with a corrugated rubber coating, which rotated at 5 revolutions per minute. The number of animals unable to remain on the rod for 2 minutes was recorded. To determine neurotoxic TD50, the statistical method of penetration by Litchfield and Wilcoxon was used. The compounds were investigated by intraperitoneal injection in doses from 400mg/kg to 1000 mg/kg.

RESULTS AND DISCUSSION

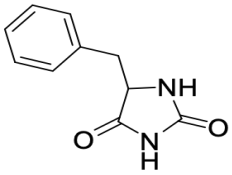
Experimental chemical part. Synthesis of hydantoins and their lithium salts: Solvents were purified by distillation before use. Crystalline compounds were recrystallized using appropriate solvents. IR spectra were recorded on a Nicolet Avatar 330 FT-IR device (Thermo Corporation, USA) in Vaseline oil. ^{1}H and ^{13}C NMR spectra were recorded on a Mercury 300 and 75 MHz spectrometer at 303 K, respectively. Chemical shifts are given relative to the internal TMS signal for DMSO-d_6/CCl_4 1:3 solution



1

To a solution of 10.5 g (0.05 mol) of benzyl and 5.25 g (0.0875 mol) of urea in 100 ml of ethyl alcohol, add 26 ml of 66% an aqueous solution of NaOH and boil the mixture for 2-3 hours. Distill to reveal the dryness solvents, add 200 ml of water, and filter off the undissolved precipitate. The clear solution is acidified with acetic acid and left in the cold. After 5 hours, the material is filtered, washed with water, and dried. Yield: 9.1 g (72%), light gray powder. After recrystallization from 70% acetic acid, the melting point is 298-300°C (293-298°C [17]). R_f 0.71 (*iso*-PrOH-dichloroethane /1:10). IR spectrum, ν, cm^{-1}. 3270, 3205 (NH), 1772, 1741, 1719 (CO), 1598 (C=C). NMR^{1}H, δ, ppm: 7.31-7.43 m (10H, 2•Ph), 9.28 s (1H, $NHCPh_2$), 11.07 br (1H, NH). NMR^{13}C, δ, ppm: 70.1 (CPh_2), 126.5 (4•CH), 127.9 (2•CH), 128.4 (4•CH), 139.9 (2•CH), 155.9 (CO), 174.7 (CO). Found, %: C 71.55; H4.83; N 11.23. $C_{15}H_{12}N_2O_2$. Calculated, %: C 71.42; H4.79; N 11.10.

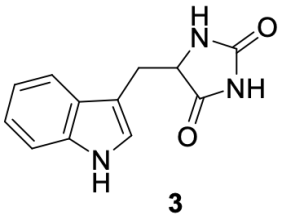
(*D,L*)-5-Benzylimidazolidine-2,4-dione. (Hydantoin (*D,L*)-β-phenyl-α-alanine) (2):



2

5.0 g (0.03 mol) (*D,L*)-β-phenyl-α-alanine is dissolved in 200 ml of water and acidified with sulfuric acid to a pH of 5.5. Then 14.6 g (0.18 mol) of crystalline KOCN is added to the solution, the solution is heated for 5 hours at 60°C, cooled to room temperature, and acidified with HCl to a pH of 4. The precipitate is filtered, washed with water, and dried. Yield: 4.1 g (72%), melting point after recrystallization from 80% AcOH is 194-196°C (194°C [18]), R_f 0.34 (*iso*-PrOH /dichloroethane / 1:10). IR spectrum, ν, cm^{-1}. 3249, 3165 (NH), 1757, 1705, (CO), 1600 (C=C). ^{1}H NMR, δ, ppm: 2.95 dd (1H, J 13.9 and 5.5 Hz, CH_2), 3.01 dd (1H, J 13.9 and 4.6 Hz, CH_2), 4.17 dd (1H, J 5.5, 4.6 and 1.2 Hz, CH), 7.15-7.28 m (5H, Ph), 7.70 br (1H, NH), 10.24 br (1H, NH). NMR^{13}C, δ, ppm 36.6 (CH_2), 58.3 (CH), 126.1 (CH), 127.5 (2•CH), 129.4 (2•CH), 135.3, 156.8, 171.3. Found, %: C 63.32; H 5.07; N 14.60. $C_{10}H_{10}N_2O_2$. Calculated, %: C 63.15; H 5.30; N 14.73.

(*D,L*)-5-((1*H*-Indol-3-yl)methyl)imidazolidine-2,4-dione. Hydantoin (*D,L*)-tryptophan (3):



3

Dissolve 6.12 g (0.03 mol) *D,L*-tryptophan in 200 ml water and acidify the pH to 5.5 with sulfuric acid. Then 14.6 g (0.18 mol) of crystalline KOCN is added to the solution. The solution is heated for 7 hours at a temperature of 60°C, after which it is cooled to room temperature and acidified with HCl to a pH of 4. Yield 4.7 g (68.4%), melting point after recrystallization from 80% AcOH is 216-218°C (215°C [19]), R_f 0.32 (*iso*-PrOH-dichloroethane/1:10). IR spectrum, ν, cm^{-1}: 3249, 3028 (NH), 1770, 1757 (CO), 1705 (C=C). NMR^{1}H, δ, ppm 3.12 dd (1H, J 14.8 and 5.5 Hz, CH_2), 3.17 dd (1H, J 14.8 and 4.3 Hz, CH_2), 4.21 dd (1H, J 5, 5, 4.3 and 1.0 Hz, CH), 6.93-6.99 m (1H), 7.00-7.06 m (1H), 7.30-7.34 m (1H) and 7.54-7.58 m (1H, C_6H_4), 7.13 d (1H, J 2,3 =CH), 7.69 br (1H, NHCH), 10.22 br (1H, NH), 10.63 br (1H, NHpyrrole). MMR^{13}C, δ, ppm 26.7 (CH_2), 58.4 (CH), 107.8, 111.0 (CH), 118.0 (CH), 118.3 (CH), 120.5 (CH), 123.8 (CH), 127.4, 135.9, 157.2, 175.1. Found, %: C 62.75; H 4.93; N 18.48. $C_{12}H_{11}N_3O_2$. Calculated, %: C 62.87; H 4.84; N 18.33.

Lithium salts of hydantoins (4-6): To 0.01 mol of hydantoins**1-3** in 20 ml of absolute methanol, add 0.07 g (0.01 mol) of lithium metal in absolute methanol, this solution is filtered in a vacuum, the methanol is distilled off, and the residue is dried in a vacuum. Light slightly hygroscopic powders are obtained and are highly soluble in water. They are stored in a vacuum dryer at room temperature.

Lithium salt of 5,5-Diphenylimidazolidine-2,4-dione (4): yield: 2.4 g (93%), m.p. 278-280°C, IR spectrum, ν, cm^{-1}: 3700-3500 (O-Li), 3204 (NH), 1771, 1740 (CO), 1717 (C=C). Found, %: C 69.67; H 4.40; N 10.73. $C_{15}H_{11}LiN_2O_2$. Calculated, %: C 69.78; H 4.29; N 10.85.

Lithium salt of (D,L)-5-Benzylimidazolidine-2,4-dione (5): yield: 1.7 g (86.7%), mp. 196-198°C. IR spectrum, ν, cm^{-1}.3700-3400 (O-Li), 3256 (NH), 1720 (CO), 1579 (C=C). Found, %: C 61.43; H 4.52; N 14.30. $C_{10}H_9LiN_2O_2$. Calculated, %: C 61.24; H 4.63; N 14.28.

Lithium salt of (D,L)-5-((1H-Indol-3-yl)methyl)imidazolidine-2,4-dione (6): yield: 1.9 g (81%), mp. 228-230°C, IR spectrum, ν, cm^{-1}: 3700-3404 (O-Li), 3275 (NH), 1693 (CO), 1574 (C=C). Found, %:C 61.50; H 4.13; N 17.70. $C_{12}H_{10}LiN_3O_2$. Calculated, %: C 61.29; H 4.29; N 17.87.

Biological research results: Studies have shown that all studied compounds exhibit elevated antagonism to both maximal electric shock and pentylenetetrazol. All compounds exhibited antagonism for nicotine and camphor convulsions. In an effective 50% dose, according to the maximal electric shock test, they are inferior to phenytoin (№1) and its lithium salt (№4), but some of them (№ 2,5,6) are superior in antipentylenetetrazol effect (Table 1). In the TSC seizure test, the compounds increase the latency period of seizures. Phenytoin at a dose of 50 mg/kg increased the latent period of seizures by 9.2 times compared to the threshold (I=1), and the tested compounds increased latency by 12 times or more. The compounds, unlike Phenytoin, are less neurotoxic and less toxic in general. Therapeutic and protective indices show that all compounds have high protective indices in the two tests and high therapeutic indices (Figure 1). Lithium chloride does not have anticonvulsant activity (40% effectiveness according to the PTZ test). The compounds are not active in the strychnine convulsions and arecoline tremor tests.

Table 1. Antagonism according to the studied tests of compounds 1-6.

Comp.№	*ANTAGONIZM*				
	According to PTZ, ED_{50}, mg/kg	***According to MES, ED_{50}, mg/kg***	***TSC (latent period of seizures), min***	***Nicotin*** **ED_{50}, mg/kg**	***Camphora*** **ED_{50}, mg/kg**
Control	-	-	12.2 ± 6.1	-*	-**
№1	35(29.2÷ 42)	17(14.2÷20.4)	112.2 ± 7.3	29.1 ± 5.2	35.0 ± 6.5
№2	20(16.7÷ 24)	40(33 ÷ 48)	149.0 ± 6.5	23.2± 4.5	28.0 ± 7.2
№3	34(19.2 ÷ 42)	87(68 ÷111.4)	157.0 ±10.3	32.5 ± 5.8	34.0 ± 8.3
№4	25(21.2 ÷29.5)	16(13.3÷ 19.2)	161.0 ±8.4	31.2 ± 7.0	35.3 ± 7.1
№5	10(8.7 ÷ 11.5)	25(21 ÷ 30)	166.0 ± 6.9	30.3 ± 4.9	29.7 ± 5.4
№6	12(10 ÷ 14.4)	20(16.6 ÷ 24)	175.0 ± 8.1	28.2 ± 5.3	30.0 ± 5.5

*Convulsions occur within 90 seconds; **Convulsions occur within 5-10 minutes.

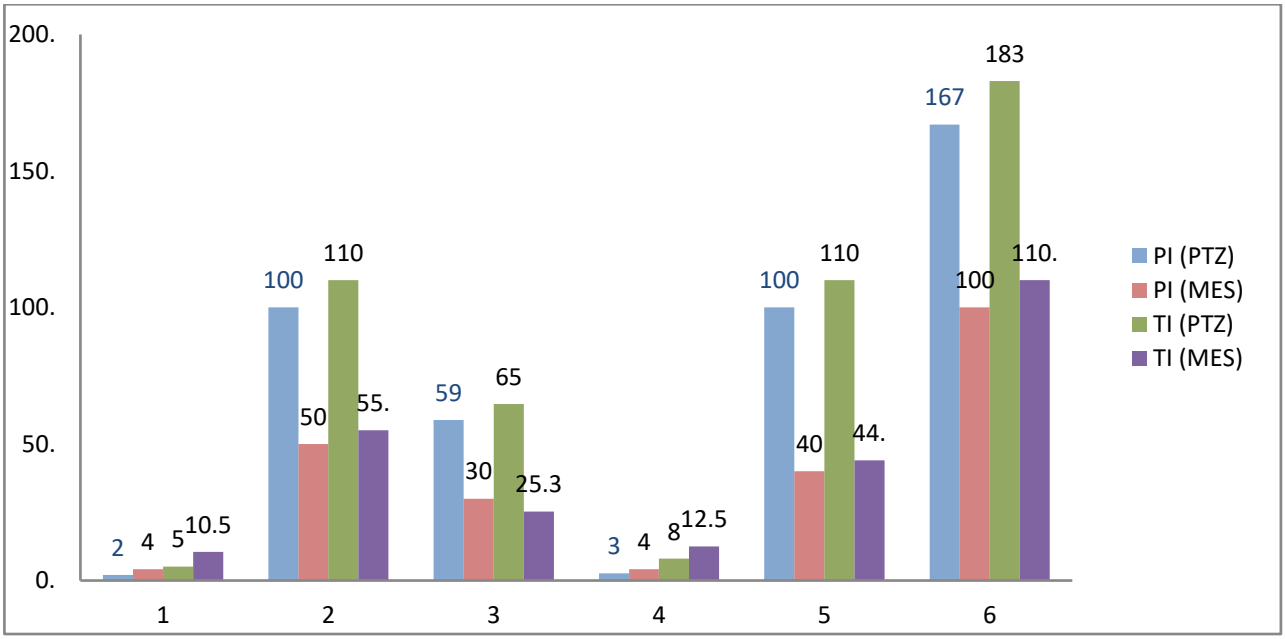


Figure 1. Protective and therapeutic indexes values of the compounds 1-6. Captions for the drawings: The abscissa axis shows the numbers assigned to the compounds studied. The ordinate axis shows the values protective and therapeutic indexes by PTZ and MES.

In the open-field exploratory activity test, compounds at doses of 50 mg/kg significantly increased the number of vertical and horizontal movements compared to the control group rats (Table 2, Figure 2). The picture is different from the action of lithium chloride. In the ''open-field'' test of exploratory activity, the number of horizontal movements in the control group rats was 32.8; vertical – 3.2 and studied cells – 2.4. Notably, in animals, all compounds lead to an increase in the number of sniffing cells; that is, the compounds exhibit anxiolytic activity. Lithium chloride does not have this effect. Thus, the study showed that the compounds, like Phenytoin, have a behaviorally activating effect and exhibit an anxiolytic effect, which is especially pronounced in derivative № 6, i.e., the lithium salt of DL-tryptophan.

Table 2. Effects of Tested Compounds on Research Activity in the ''Open Field'' Test

Compounds, Dose, 50 mg/kg	**Amount (absolute data during 5min)***		
	Horizontal displacement	**Vertical displacement**	**Sniffing the cells**
Control	32.8 ± 5.1	3. 2 ± 0.3	2.4 ± 0.2
№1	49.6 ± 5.1**	7.8 ± 2,1**	3.6 ± 0.9**
№2	46.2 ± 4.3**	10.6 ± 2.2**	3.2 ± 0.3**
№3	52.0 ± 2.5**	4.8 ± 0.8**	3.6 ± 0.7**
№4	63.6 ± 10.1**	5.2 ± 0.6**	3.8 ± 0.9**
№5	64.0 ± 10.1**	10.8 ± 2.3**	4.2 ± 0.9**
№6	68.5 ± 8.2**	11.3 ± 3.6**	10.3 ± 2.3**

*P ≤ 0.05 at a probability level; **The differences are statistically significant compared with the control.

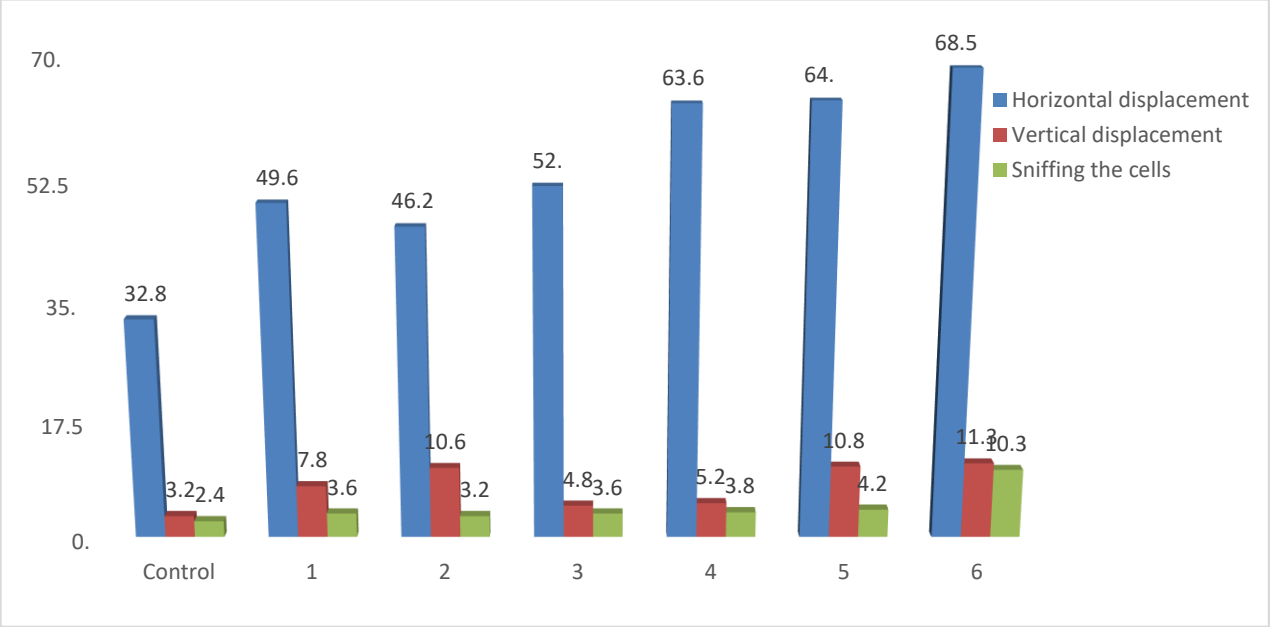


Figure 2. Effect of compounds (1-6) in rats in the ”open-field” test. The abscissa axis shows the control and numbers of the compounds studied. The ordinate axis shows the values of indicators of the model ''open-field''.

In the ''elevated plus maze'' experimental model, the administration of compounds at 25 and 50 mg/kg (more pronounced at a dose of 50 mg/kg) decreased the time spent in closed arms. The experimental model increased the time spent in open arms, in contrast to the control (Table 3, Figure 3). In the latter case, the control animals do not go into the open sleeves due to a fear of heights. An interesting pattern was observed concerning the time the mice spent in the center of the device. This rate increased after the introduction of all potential drugs, regardless of the dose used. A comparable drug, lithium chloride, lacks these properties. The data obtained allows us to think about the studied compounds’ anti-anxiety (anxiolytic) effect (including Phenytoin).

Table 3. Effects of the studied compounds in the "elevated plus maze" model (during the 5min study)

Compounds, Dose, 50 mg/kg	**Time spent in closed arms /s/***	**Time spent in the open arms /s/***	**Time spent in the center /s/***	**Number of entries into the closed arms* /s/***
Control	257.4 ± 12.5		2.6 ± 1.	19.6 ± 1.2
№1	216.0 ± 13.0 **	50.2 ± 10.2**	33.0 ± 6.2**	3.4 ± 0.4**
№2	146.0 ± 15.0**	80.0 ± 23.1**	74.0 ±11.2**	4.0 ± 1.2**
№3	227.8± 13.0**	55.4 ± 10.8**	16.8 ± 4.4**	5.0 ± 1.7**
№4	197.6 ± 12.0**	60.0 ± 10.2**	42.4 ±11.1**	6.4 ± 0.9**
№5	222.0 ± 7.5**	32.2 ± 0.5 **	45.8 ± 4.8**	9.4 ± 2.3**
№6	198.6 ± 12.8**	22.4 ± 4.2**	79.0 ± 12.5**	5.4 ± 1.4**

*P ≤ 0.05 at a probability level; **The differences are statistically significant compared with the control.

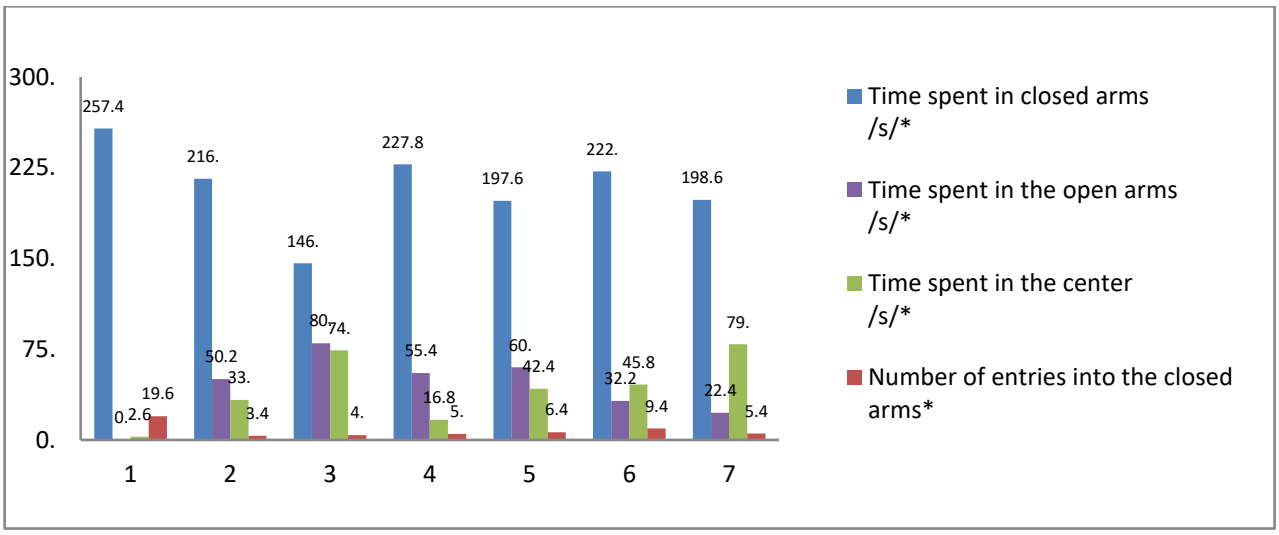


Figure 3. Effect of compounds (1-6) on the state of "fear and despair" of mice in the EPM model (observation time 5 min). The abscissa axis shows the control and numbers assigned to the compounds studied. The ordinate axis shows the values of the PCL indicators of the model.

The immobilization of the control animals under the conditions of ''forced swimming'' as a way out of "despair" is achieved quite late, specifically after 147.5 s. Administration of compounds at 50 mg/kg leads to an increase in the time of the first immobilization. This proves that the studied amino acids hydantoins and their lithium salts exhibit some antidepressant activity, in contrast to lithium chloride (Table 4).

Table 4. Effects of investigated compounds and lithium chloride in the forced swimming model (during the 6-minute study)*

Compounds, Dose 50 mg/kg	Time of active swimming (s), latent period I immobilization	Total time of immobilization (s)	Total time of active swimming (s)
Control	147.5 ± 5.2	42.8 ±4.5	317.2 ±12.2
№1	160.8 ± 9.7	35.0 ± 4.8	325.0 ± 13.2
№2	180.0 ± 10.1**	30.0 ± 4.5**	330.0 ± 12,5**
№3	120.0 ± 6.3**	49.2 ± 5.1	310.8 ± 10.2
№4	109.3 ± 5.9**	75.4 ± 6.2**	284.6 ± 9.7**
№5	195.1 ± 10.5**	80.1 ±6.6**	279.9 ± 7.8**
№6	185.0 ± 10.1**	35.0 ±4.1	325.0 ± 17.9
Lithium chloride	113.0 ± 8.5**	214.0 ±11.1**	146.0 ±9.1**

*P ≤ 0.05 at a probability level
**The differences are statistically significant compared with the control.

Under conditions of forced swimming (Table 4, Figure 4), immobilization of control animals as a way out of ''hopelessness'' is achieved quite late, specifically in 147.5 seconds. The introduction of compounds № 1,2,5,6, at a dose of 50 mg/kg, leads to an increase in the time of first immobilization. However, the total time of immobilization and the total time of active swimming change statistically insignificantly compared to the control. Under the influence of lithium chloride at a dose of 50 mg/kg and especially at a dose of 100 mg/kg, changes in the behavior of animals occur, and the onset of the first immobilization is reduced to 113 seconds and 88 seconds, respectively. The total time of immobilization increases and the total time of active swimming decreases with the compounds № 4,5. In fact, some amino acid hydantoins have shown some antidepressant effects, unlike lithium chloride.

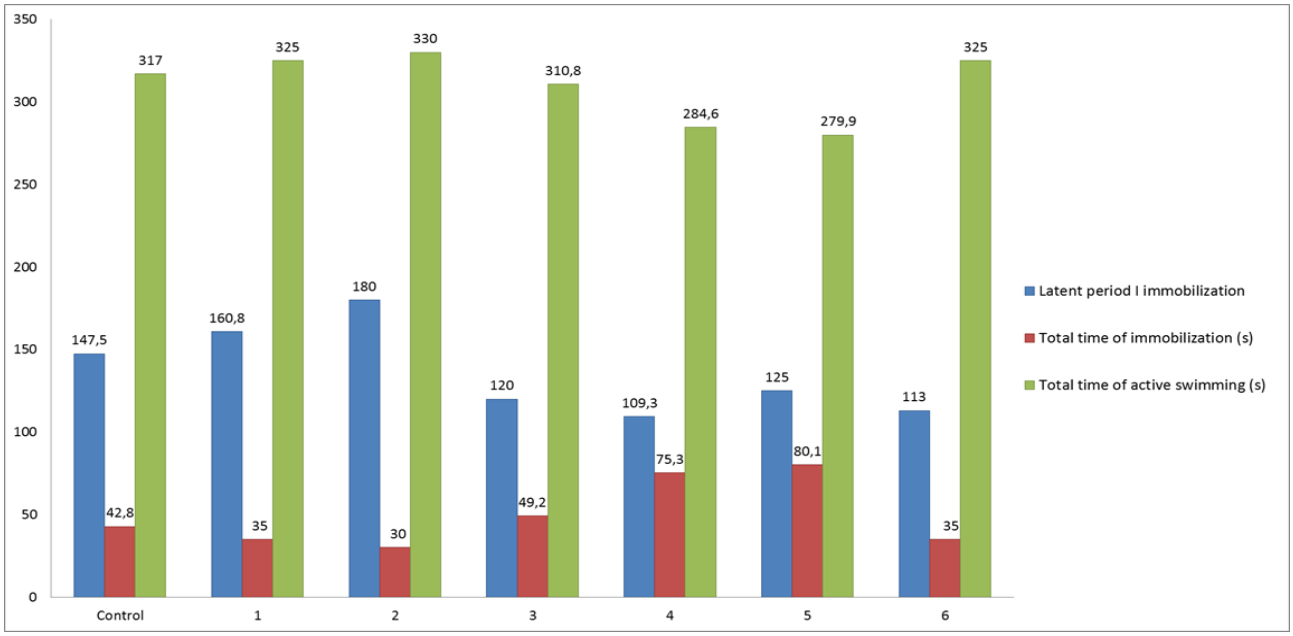


Figure 4. Effect of investigated compounds (1-6) on the "forced swimming" (observation time 6 min). The abscissa axis shows the control and numbers of the compounds studied. The ordinate axis shows the values of the forced swimming model indicators.

Studies of the activity of compounds on MAO have shown that in *in vitro* experimental conditions in the brain of rats, α-alanine hydantoin from the above compounds inhibits the deamination of serotonin by 59% at a concentration of 1 µmol/ml and 75% at a dose of 5 µmol/ml after 30 min of preincubation. For tryptophan hydantoin, deamination reaches up to 92% (1 µmol/ml). Phenytoin, on the contrary, activates the MAO enzyme (+18%, +20%) in both cases (Table 5). Phenytoin (No. 1), at a concentration of 5 µmol/ml with a 60-minute preincubation, inhibits serotonin deamination by 88%. Increasing the dose to 5 µmol/ml eliminates the activating effect. The compounds with the substrate (NA-norepinephrine) at a concentration of 1 µmol/ml at 30 and 60-minute preincubation act in the same way as serotonin and at a concentration of 5 µmol/ml for 60 minutes. Preincubations act almost like indopan (control). It is known that indopan [35] has a robust anti-MAO effect and reliably inhibits serotonin deamination, which allowed us to use this drug for comparison with the studied compounds.

The effect of the compounds on bovine liver in concentrations of 1 and 5 µmol/ml during the 30-minute and 60-minute preincubation period revealed that they have anti-MAO activity. In all samples, lithium chloride was also tested, which inhibited the deamination of serotonin in the brain and liver by 41%.

Table 5. The effect of compounds on the enzyme MAO in the brain of large-horned cattle *in vitro* conditions

Compounds	**5-OT 1 µmol/ml**		**5-OT 5 µmol/ml**		**NA 1 µmol/ml**		**NA 5 µmol/ml**	
	30'	60'	30'	60'	30'	60'	30'	60'
№1	+18	52±2,2	+20	88±2,6	+18	+22	31±1,	45±1,8
№2	59±1,2	35	75±1,8	0	62±2,4	65±2,8	43±1,5	67±2,4
№3	92±3,4	-	-	-	-	-	-	-
№4	31±1,2	0	60±2,2	0	36	45±1,2	75±2,8	83±3,0
№5	37±1.1	68±2,4	60±2,4	39±1,2	56±2,0	62±2,2	+6	+12
№6	68±1.8	75±2,4	82±2,8	90±3,2	66±2,6	58±1,8	68±2,	72±2,4
Lithium chloride	41±1,2	-	-	-	-	-	-	-

*The intensity of deamination in control samples is taken as 100%. Control of serotonin - indopan (86%), control of NA - imprazid (72%)

Thus, the compounds demonstrate moderate effects at a 30-minute preincubation and markedly expressed activity at a 60-minute preincubation in studies on the brain and liver. They can also be considered as potential antidepressants for the prevention and treatment of depressive diseases.

CONCLUSIONS

The novelty of this work is that we synthesized and studied new compounds, which display high neurotropic properties. Thus, the obtained data allowed us to conclude that the synthesized and studied hydantoins amino acids and their respective lithium salts display pronounced anticonvulsant activity (according to the tests of maximal electric shock, PTZ, nicotine, camphor, TSC convulsions, especially in the pentylenetetrazol antagonism test). They also display high psychotropic effects, as well as tranquilizer, antidepressant, and antimanic properties. According to some of their biological (neurotropic) properties, they are superior to the drugs currently used in the clinical setting, such as phenytoin, its structural analog, which was synthesized with all the hydantoin amino acids. The hydantoins of α-alanine and tryptophan, as well as their lithium salts, are superior to phenytoin and its lithium salt in terms of therapeutic and protective indices, as they are almost non-toxic (in most substances LD50>2200 mg/kg) and slightly neurotoxic (in most substances TD50 >2000 mg/kg). According to all the studies' tests, the studied compounds are superior to lithium chloride, which is used in the treatment of bipolar mental disorders. Concurrently, the compounds exhibit antidepressant and anti-MAO effects. The studied compounds can be used as antiepileptic agents with psychotropic properties and as antidepressants, especially in the treatment of manic-depressive states.

Conflict of Interest: The authors declare that the study was conducted without a potential conflict of interest.

List of Abbreviations: MAO, monoamine oxidases**;** PTZ, pentylenetetrazole; MES, maximal electroshock; TSC, thiosemicarbazide; GABA, gammaamine oxidase; NA, norepinephrine.

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