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Research Article

Design and evaluation of pharmacological properties of a new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid

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Abstract

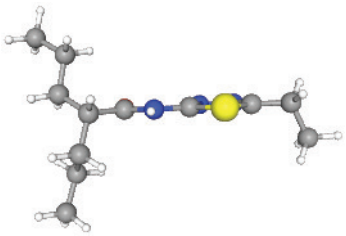
Introduction: The use of the pharmacophoric approach is a promising direction for modifying the chemical structure of 2-propylpentanoic (valproic) acid in order to obtain new drugs.

Materials and methods: In the experiments on mice, acute toxicity, neurotoxicity, antiepileptic activity and analgesic effect of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (valprazolamide) were evaluated. LD_{50} was determined by probit analysis. Neurotoxicity was determined in a rotarod test and a bar test in mice. The effects of valprazolamide on the exploratory behavior of mice in open field test and in a light/dark transition test were evaluated. Its antiepileptic activity was tested in mice against seizures induced by maximal electroshock, pentylenetetrazole (scPTZ); isoniazid, thiosemicarbazide, pilocarpine, and camphor. The analgesic effect was studied in a hot plate test.

Results and discussion: N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide was obtained by introducing pharmacophores into the structure of 2-propylpentanoic acid: a substituted amide group and an electron-donor domain of 1,3,4-thiadiazole. The LD_{50} value for intraperitoneal administration of a new 2-propylpentanoic acid: derivative to mice was 924.8 mg/kg, and the TD_{50} value in the rotarod test and the bar test were 456.7 mg/kg and 546.7 mg/kg, respectively. The suppression of orienting responses in the animals was noted when it was administered in neurotoxic doses. Valprazolamide showed the most antiepileptic activity on models of MES, scPTZ and isoniazid antagonism tests. The ED_{50} values were 138.4 mg/kg, 74.5 mg/kg, and 126.8 mg/kg, respectively. The therapeutic indices for these models of epilepsy were 6.7; 12.4; 7.3, and protective index – 3.3; 6.1 and 3.6, respectively. In the hot plate test, valprazolamide increased the latency period before a defensive response to a thermal stimulus (ED_{50} 165 mg/kg).

Conclusion: N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide is a new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid with antiepileptic and analgesic activities, which belongs to the group of low-toxic agents.

Graphic abstract



N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (3D)
LD_{50}=924.8 mg/kg (mice, intraperitoneally)
TD_{50}=456.7 mg/kg (rotarod, mice, intraperitoneally)
ED_{50}=138.4 mg/kg (MES, mice, intraperitoneally)
ED_{50}=74.5 mg/kg (scPTZ, mice, intraperitoneally)

Keywords

acute toxicity, analgesic effect, anticonvulsant activity, neurotoxicity, 2-propylpentanoic acid, pharmacophore, thiadiazole.

Introduction

Epilepsy is one of the most common and dangerous neurological diseases, requiring constant pharmacotherapy to control the development of seizures. The need for antiepileptic drugs is constantly increasing, since more than 75 million people suffer from epilepsy in the world (Avakyan et al. 2019; Beghi 2020). The prevalence of epilepsy in the Russian Federation is 3.2 per 1000 (Avakyan et al. 2017). It is in third place among neurological diseases after strokes and dementia (Gulyaev et al. 2011).

Despite the presence of a large number of basic (valproate, carbamazepine, phenytoin, topiramate, levetiracetam) and additional (rufinamide, zonisamide, retigabine, perampanel, lacosamide, etc.) antiepileptic drugs (Loizon et al. 2017), pharmacotherapy of patients with epilepsy is not always effective and safe (Nevitt et al. 2017). The frequency of drug-resistant forms of the disease is about 30% (Tang et al. 2017; Kalilani et al. 2018; Löscher 2020), so the search for new anticonvulsants remains relevant. Along with the development of original molecules, it is promising to modify the chemical structure of the known antiepileptic drugs, including valproic acid (VPA). VPA is an anticonvulsant of the simplest structure - a fatty acid with a chemical structure of 2-propylpentanoic acid. The advantage of VPA is its effectiveness in various forms of epilepsy, as well as a wide range of pharmacological activities, including antitumor, analgesic, antiretroviral, neuroprotective effects, etc. (Perucca 2012; Tomson et al. 2016). The antiepileptic effect of VPA is mediated by the inhibition of GABA transaminase and the blockade of voltage-dependent Na^+ channels in the brain, which suppresses the over-activation of neuronal cells (Piplani et al. 2016; Bertelsen et al. 2018). It has also been reported that VPA may inhibit histone deacetylase (Heers et al. 2018).

Modification of VPA with 1,3,4-thiadiazole is promising. The 1,3,4-thiadiazole fragment is an electron-donor domain due to two nitrogen atoms. The presence of a heterocyclic sulfur atom determines the high lipophilicity of thiadiazole derivatives, which gives them the ability to penetrate cell membranes easily (Haider et al. 2015). It is known that 1,3,4-thiadiazole is the basis for the creation of drugs with various pharmacological activities (Li et al. 2013; Haider et al. 2015; Abdelriheem et al. 2017; Hegab and Shamroukh 2020). A number of 1,3,4-thiadiazole derivatives have a pronounced antiepileptic activity (Raj et al. 2015). Effective anticonvulsants have been identified among derivatives of 2,5-disubstituted 1,3,4-thiadiazole (Harish et al. 2014; Toolabi et al. 2020), including those containing semicarbazone and quinazoline residues in their structure (Sharma et al. 2013). Antiepileptic activity was also reported in some derivatives of triazolo-1,3,4-thiadiazoles. The most active was 6-(4-chlorophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole, which showed anticonvulsant properties in antagonism tests with pentylenetetrazole, bicuculline, 3-mercaptopropionic acid and for seizures induced by MES (Rajak et al. 2012). In experiments on mice, antiepileptic activities were revealed in 5-butyl-2-(2,4-dihydrophenyl)-1,3,4-thiadiazole and 5-heptyl-2-(2,4-dihydrophenyl)-1,3,4-thiadiazole (Luszczki et al. 2015).

The aim of this study was to design and evaluate the pharmacological properties of a novel 1,3,4-thiadiazolylamide VPA derivative.

Materials and methods

Animals

The experimental studies on SNK white outbred male and female mice (weighing 19–23 g) were carried out. All the experiments were performed at the same time of day during daylight. All the experimental animals

were kept under 12 h light regime and in a standard animal facility with free access to water and food. All the experiments were carried out in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes (Directive 2010/53/EU). The local ethical committee approved all the protocols (Minutes No. 4, 26 March 2018).

Investigated chemical compounds

We investigated a new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid with the chemical structure N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide and the laboratory name valprazolamide (VPZ), synthesized at the All-Union Research Center for Safety of Biologically Active Substances (Staraya Kupavna, Moscow region, Russian Federation) by Professor S.Ya. Skachilova (Skachilova et al. 2018; Malygin et al. 2020). The chemical structure of VPZ is shown in Figure 1.

VPZ was identified by spectrometry (IR, NMR, mass spectrometry). Due to the presence of acidic and basic groups in its structure, mass spectra were obtained with positive and negative polarization: ESI^+-mass spectrum – *m/z* 256.1 ($[M + H]^+$) (Fig. 2), MRM transition – *m/z* 256.1 → *m/z* 81.1 and *m/z* 130.1; ESI^--mass spectrum – *m/z* 254.0 ($[M - H]^-$), MRM transition – *m/z* 254.0 → *m/z* 167.1.

The study design included the following steps:

- Evaluation of acute toxicity of VPZ after intraperitoneal injection (ip) in mice with determination of the values of lethal doses LD_{16}, LD_{50}, LD_{84} by Finney's probit analysis. Determination of the toxicity class of valprazolamide in comparison with VPA.

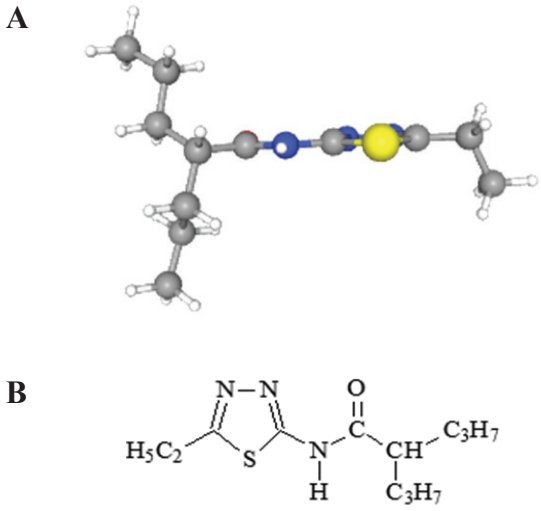


Figure 1. Chemical structure of VPZ (**A** – 3D; **B** – chemical formula).

- Evaluation of VPZ neurotoxicity in the rotarod and bar tests in mice with the calculation of the values of toxic doses TD_{16}, TD_{50}, TD_{84}, using Finney's probit analysis.
- Evaluation of the influence of VPZ on the exploratory activity of mice in the open field and light/dark transition tests.
- Evaluation of the antiepileptic activity of VPZ in maximal electroshock test (MES) and subcutaneous administration of pentylenetetrazole (scPTZ) with the calculation of the values of therapeutic doses ED_{16}, ED_{50}, ED_{84} using Finney's probit analysis. Calculation of therapeutic and protective indices for each type of activity.
- Expanded evaluation of the antiepileptic activity of VPZ in antagonism tests with pilocarpine, isoniazid, thiosemicarbazide, and camphor in mice.

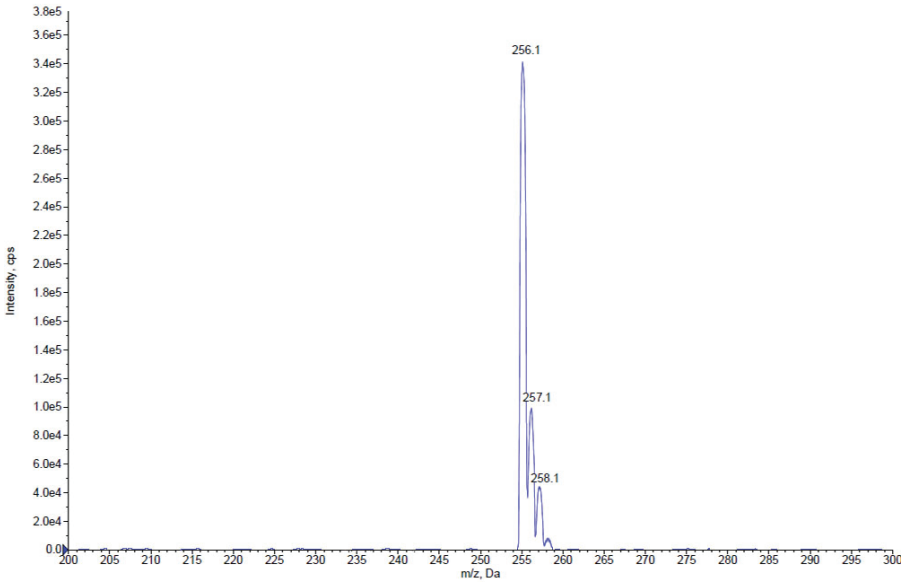


Figure 2. ESI^+-mass spectrum of VPZ. **Note:** Mass-selective quadrupole detector AB Sciex QTrap 3200 MD; detection conditions – positive polarization ($[M + H]^+$), electrospray ionization, electrospray voltage 5500.0 V, declustering potential 41.0; curtain gas pressure 20.0 psi, atomization gas pressure 40.0 psi, input potential 3.5 V. Solvent: acetonitrile: deionized water 2 : 1 with 0.1% formic acid. VPZ – N-(5-ethyl-1,3,4-thiadiazole-2- yl)-2-propylpentanamide.

- Evaluation of the anesthetic activity of VPZ in the hot plate test in mice.

All the experimental studies were performed in accordance with the Guidelines for Conducting Preclinical Studies of Drugs (2012) with generally accepted experimental models of epilepsy.

Drug administration

N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide was administered to the experimental animals intraperitoneally in Tween 80/water suspension. The volume of the administered drug did not exceed 1 ml for rats and 0.5 ml for mice.

Acute toxicity evaluation

The experimental mice were divided into 5 groups of 6 animals and received N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide at doses of 300, 600, 900, 1200, 1500 mg/kg intraperitoneally. The groups were observed for 14 days, and at the end of this period mortality was recorded for each group. DL_{50} value was determined by Finney's probit analysis. The hazard class of the studied compound was determined by the value of DL_{50} in accordance with the classification of chemicals by the parameters of acute toxicity with parenteral routes of administration (Berezovskaya 2003).

Neurotoxicity evaluation

Rotarod test

The mice were randomized into the following groups: control group (saline) and 5 experimental groups (test compound). Each group consisted of 8 animals. Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 600, 900 mg/kg) were administered intraperitoneally (i.p) 40 min before the tests.

Neurotoxicity was indicated by the inability of an animal to maintain equilibrium on the rod (with a diameter of 2.5 cm and a rotation speed of 6 rpm) for at least one minute in each of the three trials. TD_{16}, TD_{50}, TD_{84} were determined by Finney probit analysis.

Bar test

The mice were randomized into the following groups: control group (saline) and 5 experimental groups (test compound). Each group consisted of 8 animals. Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 600, 900 mg/kg) were administered intraperitoneally (i.p) 40 min before the tests.

The bar test was performed by hanging the mice with their forelimbs on a wire with a diameter of 0.4 cm, stretched at a height of 30 cm from the table surface. Neurotoxicity was indicated by the inability of an animal to pull the hind limbs to grasp the wire. TD_{16}, TD_{50}, TD_{84} were determined by Finney's probit analysis.

Behavioral tests (behavioral research)

Open field test

The mice were randomized into the following groups: control group (saline) and 6 experimental groups (test compound). Each group consisted of 8 animals. Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 450, 600, 900 mg/kg) were administered intraperitoneally (i.p) 40 min before the tests.

Observation of the experimental animals in the open field setup was carried out for 5 minutes, with the experimental animal being placed in the center of the site. The time spent in the central and peripheral squares, the numbers of intersections of the central and peripheral squares, rearings, hope peekings, boli, groomings and urinations were recorded.

Dark/light transition test

The dark/light transition test is based on the natural aversion of mice to brightly illuminated areas and on their spontaneous exploratory behavior in response to mild stressors, such as novel environment and light. The mice were randomized into the following groups: control group (saline) and 6 experimental groups (test compound). Each group consisted of 8 animals. Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 450, 600, 900 mg/kg) were administered intraperitoneally (i.p) 40 min before the tests. The experimental animals were placed in the light compartment of the test box; the number of movements between the compartments and the time spent in the dark and light compartments of the chamber were recorded during 5 minutes of observation.

Anti-epileptic models

Seizures induced by maximal electroshock (MES) in mice

Seizures were induced by electrical stimuli (50 Hz, 50 mA, duration of 0.2 s) using corneal electrodes. Maximal electroshock-induced seizures simulate Grant mal seizures. The animals had been screened before, and the mice which had showed the extension of hind limb (lasting for 3 seconds or more) upon electric shock were included in the study. All the experimental animals were instilled with eye drops with lidocaine (2%) immediately prior to the induction of maximal electroshock, and the electrodes were moistened with saline.

The mice were randomized into the following groups: control group (saline), reference drug (valproic acid 200 mg/kg) and 5 experimental groups (test compound). Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 450, 600 mg/kg) were administered intraperitoneally (i.p) 40 min before seizures induction. The ability to prevent the development of tonic extensibility of the hind limbs was taken as a criterion for antiepileptic activity. ED_{14}, ED_{50}, ED_{86} were determined by Finney's probit analysis.

Pentylenetetrazole induced seizures (scPTZ) in mice

The mice were randomized into the following groups: control group (saline), reference drug (valproic acid 200 mg/kg) and 5 experimental groups (test compound). Injections of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 450, 600 mg/kg) were administered intraperitoneally (i.p) 40 min before seizures induction. Seizures were induced by administration of pentylenetetrazole (95 mg/kg, subcutaneously) into the cervical region of the back of the neck. During the experiment, the latent period of the first generalized attack was recorded with the loss of the rollover reflex, the time of death and the number of dead animals. ED_{14}, ED_{50}, ED_{86} were determined by Finney's probit analysis. The therapeutic index and protective index were calculated according to the results of the experiment.

The therapeutic index

The therapeutic index was calculated on the basis of the results of the experiment (formula 1):

$$TI = \frac{ED_{50}}{DL_{50}} \quad (1)$$

where: TI – therapeutic index; ED_{50} is a median therapeutic dose (an antiepileptic effect is observed in 50% of the animals); DL_{50} is a median lethal dose.

The protective index

The protective index was calculated on the basis of the results of the experiment (formula 2):

$$PI = \frac{ED_{50}}{TD_{50}} \quad (2)$$

where: PI – protective index; ED_{50} is a median therapeutic dose (an antiepileptic effect is observed in 50% of the animals); TD_{50} is a median toxic dose (a neurological deficit is observed in 50% of the animals).

Analgesic activity

Hot-plate test

The experiments were performed on white outbreed SHK mice 20.1±0.4 g. The mice were divided into 4 groups of 8 animals each. The experimental animals were placed on a hot aluminum plate with a temperature of 55±0.5 °C with a maximum thermal exposure time of 30 s. The latent period of the onset of a pain response (bouncing or licking limbs) was recorded 40 minutes after the administration of N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (75, 150, 300, 600 mg/kg). The ED_{50} value was determined by Finney's probit analysis.

Biochemical studies

Determination of GABA and N- (5-ethyl-1,3,4-thiadiazol-2-yl) -2-propylpentanamide content in rat brain

The content of GABA and N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide in the brain of rats was determined by the chromatography-mass spectrometric method. The HPLS-MS-MS method was validated by the parameters of selectivity, accuracy, precision, linearity, cross-transfer and stability (EMA 2010).

Statistical analysis

A statistical analysis was performed using BioStat 2009 software (AnalystSoft; USA). The methods of descriptive statistics were used to process the research results. The type of distribution of random variables was determined using the Shapiro-Wilk *W*-test. With normal distribution of data, a one-way analysis of variance (ANOVA) was carried out, with post-hoc Tukey's HSD test. In cases of abnormal data distribution, the Kruskal-Wallis test and the Mann-Whitney U test were used. For a statistical analysis of the relative indicators, the chi-square ($\chi 2$) method was used. In cases of normal distribution, the data were presented as M±SEM, in cases of abnormal distribution – as Median (LQ–UQ). Differences were considered statistically significant at a probability level of 95% or more ($p<0.05$).

Results and discussion

Design of a new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid

The design of a new VPA derivative was carried out on the basis of a pharmacophore model. The branched hydrocarbon chain (hydrophobic domain) of VPA is the main pharmacophore that determines its antiepileptic properties (Fig. 3).

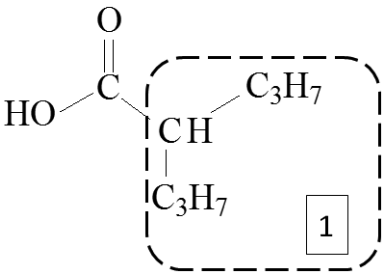


Figure 3. The pharmacophore of VPA. **Note:** 1 – hydrophobic domain of VPA.

N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide (laboratory name: VPZ – valprazolamide) was synthesized by introducing the following pharmacophores into the VPA structure: 1,3,4-thiadiazole (electron-donor domain) and substituted amide group (Fig. 4).

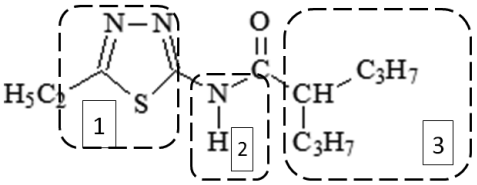


Figure 4. The pharmacophores of VPZ. **Note:** 1 – electron-donor domain, 2 – substituted amide group, 3 – hydrophobic domain.

A pharmacophoric analysis of the structure of the new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid suggested that it possesses antiepileptic activity.

Assessment of acute toxicity and neurotoxicity of VPZ

The lethal doses of VPZ (ip) and VPA (ip) were determined by probit analysis in order to assess the acute toxicity of the new 1,3,4-thiadiazolylamide VPA derivative (Table 1).

Table 1. The Values of the Lethal Doses (LD_{16}, LD_{50}, LD_{84}) of VPZ (Mice, ip) and VPA (Mice, ip)

Compound	LD_{16}, mg/kg (95% CI)	LD_{50}, mg/kg (95% CI)	LD_{84}, mg/kg (95% CI)
VPZ	764.0 (492.5÷880.3)	924.8 (756.9÷1063.7)	1119.3 (984.5÷1518.7).
VPA	430.0 (261.5÷491.3)	515.3 (420.5÷588.5)	617.3 (547.9÷869.8)

Note: The probit analysis results are presented (n=8 in each group); 95% CI – 95% confidence interval, VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, VPA – valproic acid, LD – lethal dose (LD_{16} – 16% mortality , LD_{50} – half-lethal dose, LD_{84} – 84% mortality).

The manifestations of VPZ acute toxicity were inhibition of the exploratory activity, motor activity (from mild inhibition to adynamia) and the reaction of the mice to audio, tactile, noxious, thermal, photic and other nonspecific stimuli, impaired coordination of movements, decreased muscle tone, and refusal from food and water.

The values of the lethal doses of VPZ were statistically significantly higher than that of VPA, which indicates its lower toxicity.

The values of the toxic doses were determined in rotarod and bar tests in mice using probit analysis. It was shown that when used in neurotoxic doses, valprazolamide in mice caused impaired coordination of movements, the impaired ability to hold onto a rotarod, and pull the hind limbs up onto a horizontal wire. The values of the toxic doses of VPZ (mice, ip) are shown in Table 2.

Study of the behavior of mice in the open field test and light/dark transition test

No statistically significant differences in the behavior of the animals in the open field test and dark/light transition test were found in comparison with the control when VPZ was injected intraperitoneally at the doses of 75–300 mg/kg and VPA at a dose of 150 mg/kg. VPZ at a dose of 300 mg/kg increased the exploratory activity of the mice insignificantly: the number of hole peekings increased by an average of 1.3 times ($p < 0.05$).

Table 2. The Values of the Toxic Doses (TD_{16}, TD_{50}, TD_{84}) of VPZ (Mice, ip)

Test	TD_{16}, mg/kg (95% CI)	TD_{50}, mg/kg (95% CI)	TD_{84}, mg/kg (95% CI)
Rotarod	311.3 (127.5÷402.0)	456.7 (325.4÷603.6)	669.9 (526.8÷1428.6)
Bar	452.5 (184.9÷525.2)	546.7 (443.5÷736.4)	660.4 (566.4÷1851.7)

Note: The probit analysis results are presented (n=8 in each group); 95% CI – 95% confidence interval, VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, TD – toxic dose (TD_{16} – manifestations of neurotoxicity in 16% of cases, TD_{50} – manifestations of neurotoxicity in 50% of cases, TD_{84} – manifestations of neurotoxicity in 84% of cases).

The inhibition of the motor and exploratory activities of the animals was reported at the doses of 450 mg/kg or more. There was a statistically significant inhibition of horizontal motor activity in the mice (Table 3).

Table 3. The Number of Sectors Crossed in the Open Field Test in Mice With the Administration of VPZ (ip) and VPA (ip)

Series of experiments	Dose, mg/kg	Number of sectors crossed			
		Center	2/3 of arena	Periphery	Total
VPZ	75	5.3±1.3	29.7±3.4	81.5±8.1	116.5±10.3
VPZ	150	6.7±1.4	38.5±5.3	74.5±6.7	119.7±14.1
VPZ	300	7.1±1.6	34.3±4.9	86.1±9.9	127.5±11.5
VPZ	450	1.3±0.5*	11.6±4.4*	34.5±4.3*	47.4±5.6*
VPZ	600	No	2.7±1.1*	26.1±6.4*	28.8±9.4*
VPZ	900	No	No	No	No
VPA	150	3.6±1.1	27.6±6.4	77.6±14.3	108.8±19.4
Control	–	5.1±1.2	33.2±3.0	84.2±9.3	122.4±11.5

Note: The values are expressed as M±SEM (n=8 in each group). VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, VPA – valproic acid, Control – saline. The symbol * indicates statistical significance ($p<0.05$) compared to the control group (saline), one-way analysis (ANOVA) with post-hoc Tukey's HSD test.

A decrease in the exploratory activity was confirmed by a statistically significant decrease in the number of rearings and hole peekings (Table 4).

Table 4. The Number of Nose-pokes and the Total Number of Rearings in the Open Field Test in Mice With the Administration of VPZ (ip) and VPA (ip)

Series of experiments	Dose, mg/kg	Number of nose-pokes	Total number of rearings
VPZ	75	11.1±1.9	23.0±2.1
VPZ	150	8.6±1.3	24.1±1.9
VPZ	300	12.8±1.8*	27.4±3.4
VPZ	450	4.3±1.1*	3.7±1.8*
VPZ	600	No	2.2±1.4*
VPZ	900	No	No
VPA	150	9.4±1.8	24.2±3.1
Control	–	9.5±1.4	25.6±2.3

Note: The values are expressed as M±SEM (n=8 in each group). VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, VPA – valproic acid, Control – saline. The symbol * indicates statistical significance ($p<0.05$) compared to the control group (saline), one-way analysis (ANOVA) with post-hoc Tukey's HSD test.

A statistically significant decrease in the number of transitions between the chamber compartments and a decrease in the time spent by the mice in the light chamber was reported when VPZ was administered at the toxic doses (450 mg/kg or more). (Table 5).

Table 5. The Number of Transitions Between the Compartments and the Time in the Light Chamber in the Light/Dark Transition Test With the Administration of VPZ (ip) and VPA (ip)

Series of experiments	Dose, mg/kg	Number of transitions	Time in the light chamber, s
VPZ	75	8 (7–10)	87 (79–93)
VPZ	150	10 (6–12)	97 (94–105)
VPZ	300	12 (9–14)	113 (91–115)
VPZ	450	4 (2–5)*	39 (31–47)*
VPZ	600	2 (1,3–2,8)*	12,5 (10.3–15.5)*
VPZ	900	No	No
VPA	150	12 (8–12)	136 (117–141)*
Control	–	8 (7–11)	96 (87–103)

Note: The values are expressed as Median (LQ – UQ) (n=8 in each group). VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, VPA – valproic acid, Control – saline. The symbol * indicates statistical significance (p<0.05) compared to the control group (saline), Mann-Whitney U-test.

Assessment of antiepileptic activity of VPZ

VPZ showed the highest antiepileptic activity in the models of MES, scPTZ, and isoniazid antagonism test. The effects of VPZ were dose-dependent. The values of the therapeutic doses were determined by probit analysis. Table 6 shows the ED_{50} values, therapeutic and protective indices.

Table 6. The Values ED_{50}, Therapeutic and Protective Indices of VPZ in the MES, scPTZ and Isoniazid Antagonism Tests in Mice

Epilepsy model	ED_{50}, mg / kg (95% CI)	TI	PI
MES	138.4 (97.2÷197.2)	6.7	3.3
scPTZ	74.5 (46.8÷106.4)	12.4	6.1
Isoniazid antagonism test	126.8 (65.5÷245.4)	7.3	3.6

Note: The probit analysis results are presented (n=8 in each group); 95% CI – 95% confidence interval, VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, MES – maximal electroshock, scPTZ – test with subcutaneous administration of pentylenetetrazole, ED_{50} – average therapeutic dose, TI – therapeutic index ($TI = LD_{50}/ED_{50}$), PI – protective index ($PI = TD_{50}/ED_{50}$).

The values of the therapeutic and protective indices of VPZ were higher than that of VPA (Table 7).

Table 7. The Values of ED_{50}, Therapeutic and Protective Indices of VPA in the MES and csPTZ test

Epilepsy model	ED_{50}, mg/kg (95% CI)	TI	PI
MES	234.0 (181.4÷278.1)	2.2	1.7
scPTZ	179.3 (137.8÷326.0)	2.9	2.1

Note: The probit analysis results are presented (n=8 in each group); 95% CI – 95% confidence interval, VPA – valproic acid, MES – maximal electroshock, scPTZ – test with subcutaneous administration of pentylenetetrazole, ED_{50} – average therapeutic dose, TI – therapeutic index ($TI = LD_{50}/ED_{50}$), PI – protective index ($PI = TD_{50}/ED_{50}$).

VPZ (150 mg/kg) statistically significantly increased the duration of the latent period of seizures, but did not prevent the death of the animals in antagonism tests with thiosemicarbizide (glutamate decarboxylase inhibitor), camphor (analeptic that disrupts the exchange of cerebral monoamines), and pilocarpine (M-cholinomimetic). The effects of VPZ at 150 mg/kg were comparable to those of VPA at 200 mg/kg (Table 8).

Table 8. The Effects of VPZ (150 mg/kg) and VPA (200 mg/kg) on the Duration of the Latency Period of the First Seizure Attack and the Survival of Mice With Convulsions Induced by Pilocarpine (350 mg/kg ip), Thiosemicarbazide (30 mg/kg ip), and Camphor (1000 mg/kg ip) in Mice

Series of experiments	Dose, mg/kg	LP1, min	Survival of mice, %
Saline + pilocarpine	–	6.1±1.2	37.5
VPZ + pilocarpine	150	9.1±1.4*	50
VPA + pilocarpine	200	11.4±1.7*	50
Saline + thiosemicarbazide	–	40.1±4.5	0
VPZ + thiosemicarbazide	150	64.2±7.4*	37.5
VPA + thiosemicarbazide	200	69.4±6.2*	37.5
Saline + camphor	–	4.8±1.2	0
VPZ + camphor	150	6.7±1.4*	0
VPA + camphor	200	10.4±3.2*	12.5

Note: The values are expressed as M±SEM (n=8 in each group); VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, VPA – valproic acid, LP1 – the duration of the latency period of the first convulsive seizure (the duration of the latency period before myoclonus of the head for pilocarpine). The symbol * indicates statistical significance (p<0.05) compared to the control group (saline), one-way analysis (ANOVA) with post-hoc Tukey's HSD test.

Study of analgesic activity of VPZ

In the hot plate test in the mice, VPZ (300–600 mg/kg ip) significantly increased the latency period before the defense response to a thermal stimulus. This fact indicated that VPZ had an analgesic activity. The ED_{50} value according to the results of probit analysis was 165 mg/kg. When VPZ was used at a dose of 900 mg/kg, there was no defense response during the maximum exposure time (30 s), which was a manifestation of the neurotoxic properties of the studied valproate (Fig. 5).

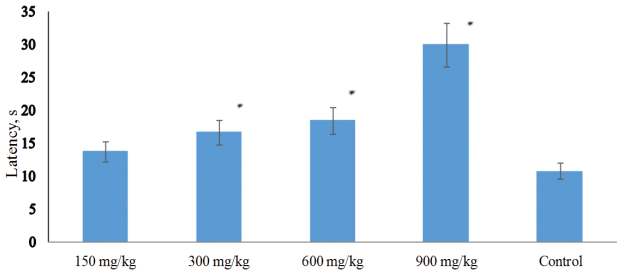


Figure 5. Effect of VPZ (ip) on the duration of the latent period before a noxious defense response in the hot plate test in mice. **Note:** The values are expressed as M±SEM (n=8 in each group); Control – saline, Latency – the duration of the latency period before the defense response to a thermal stimulus. The symbol * indicates statistical significance (p<0.05) compared to the control group (saline), one-way analysis (ANOVA) with post-hoc Tukey's HSD test.

The use of the pharmacophoric approach is a promising direction for modifying VPA to increase its effectiveness and safety (Malygin and Yasnetsov 2020). The following pharmacophores are important for an anticonvulsant activity: electron-donor domain, hydrophobic aryl or other distal hydrophobic radical, and hydrogen bonds. An α-substituted amide group may be one of the pharmacophores that determine an antiepileptic activity (Krivoshein 2016). This assumption is confirmed by the

fact that a number of amide derivatives of VPA have a high antiepileptic activity (Pessah et al. 2011; White et al. 2012; Kaufmann et al. 2017). In addition, amidation of the carboxyl group of VPA leads to a decrease in its teratogenicity (Okada et al. 2004). The data presented show the relevance of using the pharmacophore approach to the creation of new antiepileptic drugs.

The hydrophobic domain (distal alkyl radical) is the main pharmacophore that determines the antiepileptic properties of valproate. A new 1,3,4-thiadiazolylamide derivative of VPA was obtained by introducing additional pharmacophores into its structure - a substituted amide group and an electron-donor fragment (1,3,4-thiadiazole).

The aim for the preparation of new thiadiazolylamide VPA derivatives is an increase in bioavailability, a decrease in toxicity, and an increase in the breadth of therapeutic action of 1,3,4-thiadiazole derivatives as compared to the original compounds. The mesoionic nature of 1,3,4-thiadiazole promotes better drug penetration through cell membranes and interaction with biological targets (Haider et al. 2015).

N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide was characterized by a broad spectrum of the antiepileptic activity and less toxicity when compared to VPA. The LD_{50} value of VPZ (mice, i.p.) was 1.8 times higher than that of VPA. The new valproate exhibited the greatest antiepileptic activity in MES and seizures induced by convulsants, which reduce the content of GABA in the brain - the GABAA receptor antagonist pentylenetetrazole and the inhibitor of GABA synthesis isoniazid (Table 9).

The effectiveness of VPZ in seizures induced by GABA antagonists may be related to its effect on the content of neuroactive amino acids in the brain. VPZ is a lipophilic compound that penetrates well the blood-brain barrier. In experiments on rats (Malygin 2019; Malygin et al. 2019), an increase in the level of GABA in the frontal cortex, a decrease in the level of excitatory amino acids – aspartic acid and glutamic acid in the frontal cortex, and an increase in the level of the amide form of glutamic acid in the hippocampus and in the frontal cortex were found.

Based on these findings, the new 1,3,4-thiadiazolylamide derivative of 2-propylpentanoic acid can be considered as a potential antiepileptic agent with an improved safety profile.

Table 9. The Mechanism of Action of Convulsants and the Effectiveness of VPZ in Seizures Induced by Chemical Agents

Convulsant	Convulsant mechanism of action	VPZ efficacy
PTZ (95 mg/kg, sc)	$GABA_A$ receptor antagonist	Reduced mortality Prolonged latent period of seizures
Isoniazid (250 mg/kg ip)	GABA synthesis inhibitor (glutamate decarboxylase inhibitor)	Reduced mortality Prolonged latent period of seizures
Thiosemicarbazide (30 mg/kg ip)	GABA synthesis inhibitor (glutamate decarboxylase inhibitor)	Prolonged latent period of seizures
Pilocarpine (350 mg/kg ip)	M-cholinomimetic	Prolonged latent period of seizures
Camphor (1000 mg/kg ip)	An analeptic, violation of the metabolism of monoamines in the central nervous system	Prolonged latent period of seizures

Note: VPZ – N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide, PTZ – pentylenetetrazole, GABA – γ-aminobutyric acid.

Conclusion

An experimental study has shown that a new amide derivative of VPA and 1,3,4-thiadiazole with the chemical structure N-(5-ethyl-1,3,4-thiadiazol-2-yl)-2-propylpentanamide has a pronounced antiepileptic activity. According to the parameters of acute toxicity, the new anticonvulsant belongs to low-toxic substances. The value of the half-lethal dose (LD_{50}) of VPZ is statistically significantly higher than that of VPA, which indicates its lower toxicity. Similarly to other anticonvulsants, 1,3,4-thiadiazolylamide VPA derivative in toxic doses can cause disturbances in muscle tone, manifestations of neurological deficits, and inhibition of the orientation-exploratory behavior of animals. Valprazolamide reduces the lethality of animals in the MES tests, and antagonism tests with pentylenetetrazole, isoniazid, and thiosemicarbazide. The therapeutic doses (ED_{50}, ED_{16}, ED_{84}) were determined for each type of the antiepileptic activity by probit analysis, and the values of the therapeutic and protective indices were calculated. The analgesic activity of the studied valproate was revealed in the hot plate test in mice.

Conflict of interests

The authors declare no conflict of interests.

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