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The study of antidepressant properties of new 1,2,3-triazolo-1,4-benzodiazepine derivatives

Anxiety-depressive disorders are among the most common mental health conditions and often cause significant functional impairment, affecting a person's quality of life. Research conducted in recent years indicate the importance of studying and searching for new substances based on benzodiazepines, in particular triazolobenzodiazepines, for the treatment of anxiety states and disorders, as well as determining the presence of other biological activities of these compounds.

Aim. To study the antidepressant activity of new derivatives of 1,2,3-triazolo-1,4-benzodiazepines in the Porsolt forced swim and tail suspension tests.

Materials and methods. The antidepressant activity of new 1,2,3-triazolo-1,4-benzodiazepine derivatives under the code MA-252, MA-253, MA-254, MA-255 and MA-261 was studied in the Porsolt forced swim and tail suspension tests. The following behavioral reactions, such as the latent period of the first immobility (more than 5 seconds), the total duration of immobility (staying in a stationary state), the number of immobile episodes, were recorded.

Results and discussion. During the study, a decrease in the total duration of immobility, the main indicator of "despair" of animals, and an increase in the latent period of the first immobility were monitored. It may indicate the manifestation of antidepressant properties of new 1,2,3-triazolo-1,4-benzodiazepine derivatives. The indicator of the antidepressant activity in groups of animals administered MA-253, MA-254 and MA-255 derivatives in all doses was higher among the groups studied. The depression index was the lowest when MA-253 and MA-254 derivatives were used in the dose of 1 mg/kg, and was not statistically significantly different from that in the group receiving imipramine in the dose of 25 mg/kg. According to the results of the tail suspension test, 1,2,3-triazolo-1,4-benzodiazepine derivatives MA-253, MA-254 and MA-255 showed a significant decrease in the total duration of immobilization by 69.4 %, 47.1 % and 33.1 %, respectively, in relation to the control group ($p<0.05$), as well as an increase in the latent period of the onset of the first immobility episode by several times.

Conclusions. A decrease in the duration of immobility in mice injected with 1,2,3-triazolo-1,4-benzodiazepine derivatives gives grounds to draw a conclusion that animals develop a state of "behavioral despair" and exhibit antidepressant properties.

Keywords: *triazolobenzodiazepine derivatives; forced swim test; tail suspension test; antidepressant activity*

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Дослідження антидепресивних властивостей нових похідних 1,2,3-триазоло-1,4-бензодіазепінів

Тривожно-депресивні розлади є одними з найпоширеніших станів психічного здоров'я і часто викликають значні функціональні порушення, впливаючи на якість життя людини. Дослідження останніх років свідчать про важливість вивчення та пошуку нових речовин на основі бензодіазепінів, зокрема триазолобензодіазепінів, для лікування тривожних станів та розладів, а також виявлення інших біологічних активностей цих сполук.

Метою роботи було дослідити антидепресивну активність нових похідних 1,2,3-триазоло-1,4-бензодіазепінів у тестах примусового плавання за Порсолтом та «підвішування за хвіст».

Матеріали та методи. Антидепресивну активність нових похідних 1,2,3-триазоло-1,4-бензодіазепінів під шифром МА-252, МА-253, МА-254, МА-255 та МА-261 досліджували в тестах примусового плавання за Порсолт та «підвішування за хвіст». Реєстрували такі поведінкові реакції, як: латентний період першого зависання (понад 5 секунд), загальна тривалість іммобілізації (перебування в нерухомому стані), кількість зависань.

Результати та їх обговорення. Під час дослідження відстежували зменшення загальної тривалості завмирання, основного показника «відчаю» тварин, та збільшення латентного періоду першого зависання, що може свідчити про прояв антидепресивних властивостей нових похідних 1,2,3-триазоло-1,4-бензодіазепінів. Показник антидепресивної активності в групах тварин, яким вводили МА-253, МА-254 та МА-255 в усіх досліджуваних дозах, був вищий серед досліджуваних груп. Індекс депресивності був найнижчий за застосування похідних МА-253 та МА-254 в дозі 1 мг/кг та статистично значуще не відрізнявся від такого в групі, тварини якої отримували іміпрамін в дозі 25 мг/кг. За результатами тесту «підвішування за хвіст» похідні 1,2,3-триазоло-1,4-бензодіазепінів МА-253, МА-254 та МА-255 продемонстрували помітне зменшення загальної тривалості іммобілізації на 69,4 %, 47,1 % та 33,1 % відповідно, якщо порівнювати з групою контролю ($p<0,05$), а також збільшення латентного періоду настання першого завмирання в декілька разів.

Висновки. Зменшення тривалості іммобілізації в мишей, яким вводили похідні 1,2,3-триазоло-1,4-бензодіазепінів, дає підставу виснувати про зменшення розвитку у тварин стану «поведінкового відчаю» та прояв антидепресивних властивостей.

Ключові слова: *похідні триазолобензодіазепіну; тест примусового плавання; тест «підвішування за хвіст»; антидепресивна активність*

Introduction. Anxiety-depressive disorders are among the most common mental health conditions and often cause significant functional impairment, affecting a person's quality of life [1]. Over the past four years, the COVID-19 pandemic has had a strong impact on mental health around the world due, for example, to social isolation, economic uncertainty and health problems, which may have exacerbated anxiety symptoms in many people and triggered mental health problems [2]. The full-scale war in Ukraine combined with the post-Covid situation creates the conditions for a growing burden of mental disorders in the healthcare sector. According to the WHO, every fifth person affected by the war is at risk of developing a mental illness, which means that there are about 8.5 million such people in Ukraine today [3].

Benzodiazepines, such as diazepam, clonazepam, chlordiazepoxide, flumazenil, alprazolam, and others, are a well-known therapeutic class of diazepines commonly used in clinical practice to relieve symptoms of anxiety and depression, as well as analgesics, anticonvulsants, and hypnotics [4]. Patients diagnosed with depression are often prescribed benzodiazepines for the short period (not exceeding 4 weeks) to reduce the manifestation of anxiety and insomnia [5, 6]. In contrast to the majority of antidepressants, benzodiazepines exhibit a much quicker onset of the therapeutic action though with a shorter duration of the effect [7]. The clinical use of benzodiazepine derivatives is restricted due to the prevalence of adverse effects, including central nervous system depression, along with the potential impairment of cognitive functions [8]. Research conducted in recent years indicates the importance of studying and finding new substances based on benzodiazepines, in particular triazolobenzodiazepines, due to their effectiveness for the treatment of anxiety states and disorders with lower application risks, as well as establishing the presence of other biological activities of these compounds [9-11].

In individuals diagnosed with depression, the most common symptoms include feelings of hopelessness or helplessness, often accompanied by a loss of motivation and anhedonia, which is the lack of pleasure or interest in activities that were once enjoyable [12]. The Porsolt forced swim test and the tail suspension test are commonly are commonly used in experiments to evaluate predictive models of depressive-like behavior in mice. These behavioral tests are designed to assess the response of mice to stressful situations and are frequently utilized in preclinical research to study depressive-like symptoms and potential interventions. In these tests, the duration of immobility or passive behavior is often measured, and the increased immobility time is considered indicative of a depressive-like state in rodents [13, 14].

The preliminary studies of the pharmacological activity of new 1,2,3-triazolo-1,4-benzodiazepine derivatives showed a pronounced anxiolytic activity in the elevated plus maze and dark-light chamber tests, with the absence of a negative effect on coordination of movements and a slight muscle relaxant effect indicating a promising further study of their effects [15, 16].

The **aim** of this work was to study the antidepressant activity of new derivatives of 1,2,3-triazolo-1,4-benzodiazepines in the Porsolt forced swim and tail suspension tests.

Materials and methods. New 1,2,3-triazolo-1,4-benzodiazepine derivatives under the code MA-252, MA-253, MA-254, MA-255 and MA-261 were synthesized under the supervision of Prof. V. A. Chebanov [17]. The benzodiazepine derivatives studied were mixed (triturated) with lactose in a ratio of 1:1000 for the preliminary study of the pharmacological activity.

The study was conducted on random-bred albino mice (weighting 20-40 g), they were kept in the vivarium of the Central Research Laboratory of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy. Rodents were kept in a well-ventilated room with a 12-hour light/dark cycle and free access to food and water. Mice were randomly divided into groups of 6 animals each: the control group, which received the corresponding volume of distilled water; 15 experimental groups that were administered an aqueous solution of a 1,2,3-triazolo-1,4-benzodiazepine derivative in the doses of 0.5 mg/kg, 0.75 mg/kg and 1 mg/kg; as well as a comparison group, its animals were given the classic antidepressant imipramine in the dose of 25 mg/kg. The interval between the drug administration and the beginning of the study was 1 hour for each mouse.

The experiments were carried out in accordance with the "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes" (Council Directive 2010/63/EU, 2010), and the study was approved by the Bioethics Commission of the National University of Pharmacy (protocol No. 4 from 02.10.2020) [18, 19].

The antidepressant activity were studied using the "despair" behavioral test designed to create a stressful state in experimental animals by the Porsolt forced swim test [13, 20, 21]. Mice were placed one by one in a narrow cylinder with a diameter of 10 cm and a height of about 25 cm, filled for 1/3 with water with a temperature of 20-24 C, so that the animal did not touch the bottom with its hind legs. The animal was observed for 5 minutes, during which behavioral reactions, such as the latent period of the first immobility (more than 5 seconds), the total duration of immobility (staying in a stationary state) interpreted as manifestation of depression were recorded. The depression index was calculated as the

ratio of the number of immobility episodes to the number of active swimming periods [22]. The antidepressant activity was determined by the formula:

$$AA, \% = \frac{Tc - Te}{Tc} \times 100 \% \quad (1)$$

where Tc – is the average value of the duration of immobility of animals from the control group; Te – is the average value of the duration of immobilization of experimental animals.

The tail suspension test is a "dry" analog of the Porsolt forced swim test and as a model of despair behavior is used to study the antidepressant activity [13, 23]. Usually, to reduce the risk of injury to animals in the test, mice are used, which are lighter in body weight than rats. The rodents were fixed by the tip of the tail to the tripod with the help of a patch so that the distance from the surface of the table to the nose of the animal was about 10 cm. The duration of immobility characterized by motionless hanging of the animal, and the latent period of the first immobility (the time interval from the beginning of the experiment when the animal actively tries to escape, to the transition to a stationary position) were recorded for 5 min. In addition, the number of episodes of immobility was counted. An increase in the total duration of immobility and the duration of the onset of the first immobility episode indicates the presence of antidepressant properties in the compound studied.

The results were presented as a mean value with standard deviation. The difference between groups was analyzed using Student's test (in case of normal distribution). Changes at $p<0.05$ were considered statistically significant.

Results and discussion. The Porsolt forced swim test is the most common and most frequently used in preclinical studies of depression. It is based on the animal's desire to avoid a stressful situation, which is manifested by a decrease in the time spent in a stationary position in a cylinder filled with water. Moreover, this test is capable of detecting the antidepressant effect of APIs under conditions of acute administration, which allows for a quick assessment of their effectiveness [14, 24].

The results obtained in the forced swim test are shown in Table 1. In general, during the study, a decrease in the total duration of immobility, the main indicator of "despair" of animals, and an increase in the latent period of the first immobility episode were monitored. This may indicate the manifestation of antidepressant properties of new 1,2,3-triazolo-1,4-benzodiazepine derivatives. The indicator of the antidepressant activity determined by formula (1) in groups of animals administered MA-253, MA-254 and MA-255 in all doses was higher among the groups studied. With an increase in the dose, the growth of the effect was monitored, and at the maximum dose it was 73 % (MA-253), 71 % (MA-254) and 74 % (MA-255) in relation to the control group ($p < 0.05$). The use of the classic antidepressant imipramine prolonged the latent period of the onset of the first immobility of the animal and significantly reduced the total duration of immobility ($p < 0.05$).

The depression index (Fig.) determined by the ratio of the number of immobility episodes to the number of active swimming periods was the lowest when MA-253 and MA-254 derivatives were used in the dose of 1 mg/kg, and was not statistically significantly different from that in the group receiving imipramine in the dose of 25 mg/kg.

Table 1

The effect of the derivatives studied on the parameters of the Porsolt forced swim test (M ± m, n=6)

Animal group	Dose, mg/kg	The latent period of the first immobility episode, s	The total duration of immobility, s	AA, %
Control	-	79.5 ± 1.6	199.3 ± 2.6	-
MA-252	0.5	90.2 ± 1.4 #	168.7 ± 2.2 *,#	15 %
	0.75	89.2 ± 1.6 #	153.3 ± 3.9 *,#	23 %
	1.0	94.3 ± 1.1 *	119.7 ± 1.3 *,#	40 %
MA-253	0.5	94.0 ± 1.1 *	88.7 ± 2.5 *,#	56 %
	0.75	125.7 ± 1.2 *,#	65.2 ± 2.3 *,#	67 %
	1.0	126.2 ± 1.2 *,#	54.7 ± 2.3 *	73 %
MA-254	0.5	107.7 ± 1.4 *	77.7 ± 3.5 *,#	61 %
	0.75	111.8 ± 1.2 *,#	66.2 ± 0.8 *,#	67 %
	1.0	119.7 ± 1.5 *,#	52.5 ± 3.0 *	71 %
MA-255	0.5	117.5 ± 1.2 *,#	60.7 ± 2.5 *	70 %
	0.75	122.3 ± 1.1 *,#	53.3 ± 1.3 *	73 %
	1.0	125.0 ± 0.9 *,#	51.5 ± 3.6 *	74 %
MA-261	0.5	86.2 ± 1.3 #	117.8 ± 3.0 *,#	41 %
	0.75	86.7 ± 1.6 #	65.7 ± 1.3 *,#	67 %
	1.0	78.3 ± 1.3 #	75.5 ± 2.4 *,#	62 %
Imipramine	25.0	101.3 ± 1.3 *	47.8 ± 0.9 *	76 %

* Significant at $p < 0.05$ compared to the control group; # Significant at $p < 0.05$ compared to the group treated with imipramine.

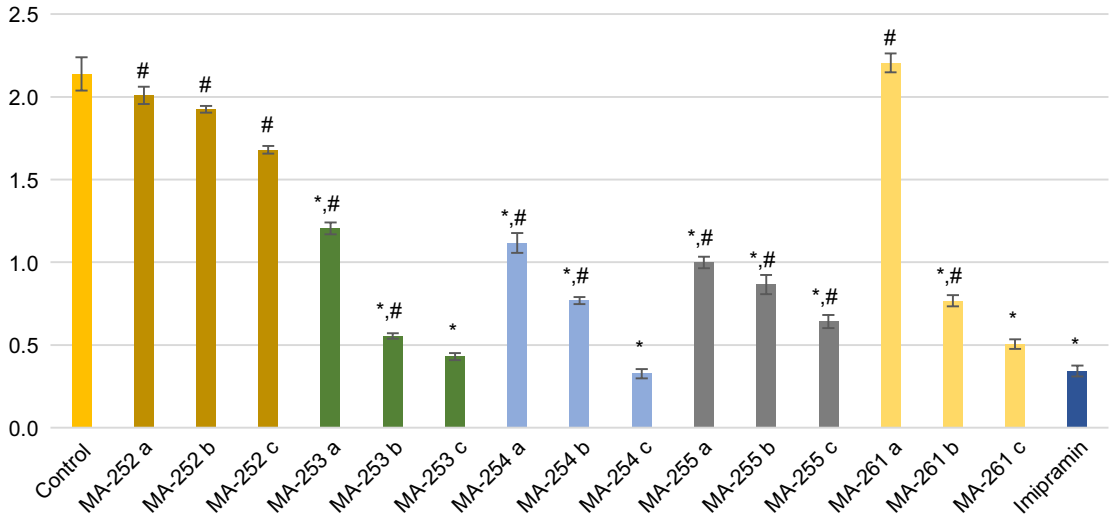

Fig. The index of depression of animals in the Porsolt forced swim test

Notes: The drug dose used was (a) 0.5 mg/kg, (b) 0.75 mg/kg, and (c) 1 mg/kg.
* Significant at $p < 0.05$ compared to the control group; # Significant at $p < 0.05$ compared to the group treated with imipramine.

Cryan and the authors systematized data from studies of various antidepressants and other classes of psychotropic compounds in the Porsolt forced swim test where they noted that, for example, diazepam in various doses under conditions of acute administration did not affect the duration of immobility, moreover, in certain doses, it even prolonged it [14]. The absence of the antidepressant effect of diazepam in the dose of 22 mg/kg was observed by Methuku and colleagues, however, in the presence of β-carboline-3-carboxylate-tert-butyl ester, diazepam significantly reduced immobility time like the tricyclic antidepressant imipramine [25]. Studies by El Zahaf and Elhwuegi showed that imipramine caused a significant dose-dependent reduction in duration by 56.3 % in relation to the control group. In turn, administration of doses of diazepam, vigabatrin, and zolpidem significantly increased the duration of immobility, and alprazolam in the dose of 5 mg/kg statistically significantly reduced the duration of immobility (74.9 % of the control group) [26]. Cardenas and authors investigated the anxiolytic- and antidepressant-like effects of an aqueous extract of *Tanacetum parthenium* L. Schultz-Bip in mice where they compared it with alprazolam in the doses of 0.03 and 0.062 mg/kg and showed that this benzodiazepine

Table 2

The effect of the derivatives studied on the behavior of animals in the tail suspension test (M ± m, n=6)

Animal group	Dose, mg/kg	The latent period of the first immobility episode, s	The number of immobility episodes	Duration of immobility, s
Control	-	10.5 ± 1.2	11.8 ± 0.4	195.2 ± 2.1
MA-252	0.5	28.7 ± 0.6 *,#	7.8 ± 0.3 *	183.7 ± 1.9 #
	0.75	48.3 ± 2.7 *,#	9.0 ± 0.3 *	179.8 ± 4.6 #
	1.0	29.2 ± 0.9 *,#	11.0 ± 0.4 #	175.8 ± 1.0 *,#
MA-253	0.5	29.8 ± 1.4 *,#	10.5 ± 0.4 #	151.8 ± 4.3 *,#
	0.75	47.3 ± 2.2 *,#	8.2 ± 0.3 *	111.2 ± 2.9 *,#
	1.0	55.5 ± 0.9 *,#	6.2 ± 0.6 *	59.8 ± 3.7 *
MA-254	0.5	24.8 ± 1.9 *,#	16.8 ± 0.5 *,#	123.7 ± 1.8 *,#
	0.75	29.8 ± 2.1 *,#	11.5 ± 0.6 #	104.8 ± 1.4 *,#
	1.0	49.0 ± 1.5 *,#	8.0 ± 0.4 *	103.2 ± 1.4 *,#
MA-255	0.5	39.2 ± 2.2 *,#	15.0 ± 0.7 #	136.8 ± 2.4 *,#
	0.75	24.8 ± 0.4 *,#	15.7 ± 0.3 *,#	135.5 ± 3.0 *,#
	1.0	37.2 ± 1.4 *,#	16.5 ± 0.6 *,#	132.5 ± 1.1 *,#
MA-261	0.5	16.0 ± 0.6 #	13.3 ± 0.4 #	148.8 ± 1.2 *,#
	0.75	21.3 ± 0.5 *,#	22.5 ± 0.7 *,#	181.0 ± 2.7 #
	1.0	20.5 ± 0.7 *,#	26.3 ± 0.8 *,#	176.8 ± 2.2 *,#
Imipramine	25.0	74.2 ± 1.3 *	6.3 ± 0.4 *	45.3 ± 1.1 *

* Significant at $p < 0.05$ compared to the control group; # Significant at $p < 0.05$ compared to the group treated with imipramine.

derivative significantly reduced the immobility time during the forced swim test compared to the control group [27].

The tail suspension test confirms the hypothesis that an animal in an intractable situation exhibits two types of behavior: anxiety and immobility, i.e. search behavior characterized by the intense motor activity and energy expenditure, and the expectant behavior with immobility and energy conservation. Antidepressants are thought to shift the balance between these behaviors in favor of seeking [28]. The main aspect of the test is the behavioral assessment of feelings of hopelessness or helplessness regarding the loss of motivation to avoid an aversive situation, which corresponds to a depressed state in a person [12].

According to the results of the tail suspension test (Table 2), the 1,2,3-triazolo-1,4-benzodiazepine derivatives – MA-253, MA-254 and MA-255 demonstrated a noticeable decrease in the total duration of immobility by 69.4 %, 47.1 % and 33.1 %, respectively, in relation to the control group ($p<0.05$), as well as an increase in the latent period of the onset of the first immobility episode by several times. The introduction of imipramine in the dose of 25 mg/kg, in turn, led to a reduction in the period of immobility by 77.7 % compared to the indicator of the group of control animals ($p<0.05$).

Hal et al. synthesized new derivatives of 2,3-dihydro-1H-1,5-benzodiazepines and studied their antidepressant potential where compounds 2 and 5 with chloro- and nitro-substituents showed a significant reduction in the immobility time of animals as assessed by the forced swim and tail suspension tests [4]. The administration of diazepam (0.1 mg/kg) and agmatine (0.0001 mg/kg) showed a synergistic antidepressant effect affecting the duration of immobility in rats, while the administration of diazepam alone had no effect [29]. The behavior of animals treated with imipramine in the dose of 15 mg/kg did not differ from the control group. The coadministration of 3,7-dimethyl-1-propargylxanthine, a caffeine analog, in the dose of 3 mg/kg with imipramine caused a reduction in immobility time in both behavioral tests [9].

The GABAergic system is involved in the pathophysiology of depression and anxiety. The reduction in the duration of immobilization in the forced swim and tail suspension tests may be explained by a possible action on α2-adrenergic receptors, which leads to an increase in the release of 5-HT, causing the antidepressant effect [26, 30]. Thus, substances that can have a positive allosteric effect on the GABAergic system can be useful in the treatment of anxiety-depressive conditions [31].

Conclusions and prospects for further research. A decrease in the duration of immobility in mice injected with 1,2,3-triazolo-1,4-benzodiazepine derivatives gives grounds to draw a conclusion that animals develop a state of "behavioral despair" and exhibit antidepressant properties. In particular, during the Porsolt forced swim test, it was found that MA-253, MA-254 and MA-255 derivatives in the doses of 1 mg/kg reduced the total duration of immobility and had a low depression index. A similar trend was observed in the tail suspension test where the above-mentioned compounds reliably prolonged the latent period of the first suspension and shortened the total period of the immobile state.

Therefore, it is advisable to further study the pharmacological effects of new 1.2.3-triazolo-1.4-benzodiazepine derivatives and determine their mechanisms of action.

Conflict of interests: authors have no conflict of interests to declare.

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