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Investigation of actoprotective activity in a range of 6-*N*-R-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones

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Key words: 6-*N*-R-Tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones, Actoprotective Activity, Normothermia, Hypothermia.

The pace of life in the XXI century often leads to mental and physical fatigue, which can be neutralized by drugs, namely actoprotectors.

Aim. To find compounds able to increase physical endurance among 6-*N*-R-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones, which are promising in this regard.

Methods and results. Actoprotective activity was tested on Wistar white rats by swimming test with the extra weight. It was found, that compounds, intragastrically administrated in 50 mg/kg, increased the duration of swimming in conditions of normothermia and hypothermia.

Conclusion. Rats' swimming duration compared with control has increased for compounds **1**, **4** and **7** to 55.50%, 42.36% and 22.92% against 22.20% of «Mildronate». The leading compound was revealed, namely, 6-methyltetrazolo[1,5-*c*]quinazolin-5(6*H*)-one, which exceeded the activity of reference drug in the normothermia and hypothermia. And it has been established, that sulfur in the 5th position of tetrazolo[1,5-*c*]quinazoline is a key element in exhibiting the actoprotective activity.

Дослідження актопротекторної активності в ряду 6-*N*-R-тетразоло[1,5-*c*]хіназолін-5(6*H*)-онів

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Темп життя людини XXI століття часто призводить до розумового та фізичного стомлення, котре можливо успішно нівелювати за допомогою лікарських засобів, а саме актопротекторів. З метою пошуку речовин з актопротекторною активністю протестували перспективні в цьому напрямі 6-*N*-R-тетразоло[1,5-*c*]хіназолін-5(6*H*)-они на білих щурах лінії Вістар за тестом плавальної проби з додатковим навантаженням. Встановили, що сполуки, котрі досліджували, при внутрішньошлунковому введенні в дозі 50 мг/кг збільшують тривалість плавання в умовах нормотермії та гіпотермії. Тривалість плавання щурів у порівнянні з контролем зросла для сполук **1**, **4** та **7** на 55,50%, 42,36% та 22,92% проти 22,20% для Мілдронату. Виявлена сполука-лідер, а саме 6-метилтетразоло[1,5-*c*]хіназолін-5(6*H*)-он, котра за активністю перевищила препарат порівняння як в умовах нормотермії, так і гіпотермії. Встановили, що сірка в п'ятому положенні тетразоло[1,5-*c*]хіназоліну є ключовим елементом у прояві актопротекторної активності.

Ключові слова: 6-*N*-R-тетразоло[1,5-*c*]хіназолін-5(6*H*)-они, актопротекторна активність, нормотермія, гіпотермія.

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Исследование актопротекторной активности в ряду 6-*N*-R-тетразоло[1,5-*c*]хинозолин-5(6*H*)-онов

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Темп жизни человека XXI столетия нередко приводит к умственному и физическому утомлению, которое возможно успешно нивелировать с помощью лекарственных средств, а именно актопротекторов. С целью поиска веществ с актопротекторной активностью были протестированы перспективные в этом направлении 6-*N*-R-тетразоло[1,5-*c*]хинозолин-5(6*H*)-оны на белых крысах линии Вистар посредством теста плавательной пробы с дополнительной нагрузкой. Было установлено, что исследуемые соединения при внутрижелудочном введении в дозе 50 мг/кг увеличивают продолжительность плавания в условиях нормотермии и гипотермии. Продолжительность плавания крыс по сравнению с контролем возросла для соединений **1**, **4** и **7** на 55,50%, 42,36% и 22,92% против 22,20% для Милдроната. Обнаружено соединение-лидер, а именно 6-метилтетразоло[1,5-*c*]хинозолин-5(6*H*)-он, которое по активности превысило препарат сравнения как в условиях нормотермии, так и гипотермии. Установлено, что сера в пятом положении тетразоло[1,5-*c*]хинозолина является ключевым элементом для проявления актопротекторной активности.

Ключевые слова: 6-*N*-R-тетразоло[1,5-*c*]хинозолин-5(6*H*)-оны, актопротекторная активность, нормотермия, гипотермия.

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Introduction. Professional activity of modern man is often carried out in conditions of prolonged influence of adverse physical and chemical factors, constant emotional and physical stress. The high pace of life typical for modern man, always leads to decrease, and often to exhaustion of physical and mental strength. Chronic fatigue syndrome has become typical for people, especially for those, who live in big city. It is characterized by decrease in systemic defense mechanisms of adaptation, immunity and ability to work. This determines the need for pharmacological treatment for homeostasis maintaining of the body in extreme conditions, which stimulates locomotor activity, capacity for work and reduces fatigue. Medicines with such action are called actoprotectors and antihypoxants [1].

The range of these drugs is unfortunately very limited nowadays (especially synthetic compounds) and existing drugs have drawbacks and side effects.

In cooperation with the Vinnytsia National M. I. Pirogov Memorial Medical University, previously synthesized 5-*R*-thio-tetrazolo[1,5-*c*]quinazolines have been already tested for actoprotective activity. Results of the study by swimming test with an extra weight and retention on the rotating rod in some cases exceeded reference drug «Bemithylum» [2,3]. Regarding the tetrazol ring, it should be noted, that a number of tetrazol containing compounds, namely 1-(1,2,3,4-tetrazol-2-yl-diazanyl)-4-substituted benzyldieneaminobenzenes, complex copper pentaaminotetrazoles, 2-{[2-(1*H*-tetrazole-5-yl)ethyl]

sulfanyl}-1,3-benzimidazoles, steroidal tetrazoles and 5-phenyl-1-(5-phenyl)-isoxazol-3-yl)-1*H*-tetrazoles also have demonstrated antioxidant activity [4–9]. It is very important, because processes of lipid peroxidation are of a great value for energy processes, cell division and the synthesis of actoprotectors.

So, in terms of insignificant number of used actoprotectors, and at the same time very high demands for such medicines, the search for new actoprotectors is an urgent task.

Thus, **the aim** of this study, was to find new actoprotectors among the synthesized 6-*N*-*R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones, to establish correlation between their chemical structure and pharmacological activity.

Materials and methods

6-*N*-*R*-Tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones were synthesized at the Department of Organic and Bioorganic Chemistry, Zaporizhzhia State Medical University (the Head of the Department, Prof., Dr. Kovalenko S. I.). The basic structure of these compounds is shown in *Figure 1*.

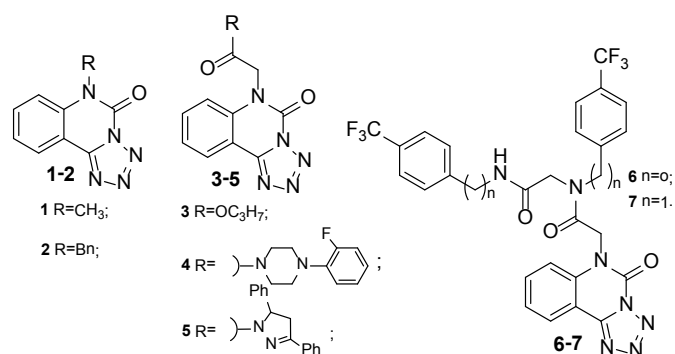


Fig. 1. The structures of tested 6-*N*-*R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones.

78 Wistar white rats, weighing 160–200.0 g, of 3.5 months age were used for the assessment of actoprotective activity of the synthesized compounds that were obtained from the nursery «BioModelServis» (Kyiv). The duration of quarantine of animals was 14 days. During this period the animals were observed twice a day. Cages of animals were placed in separate rooms, with the natural change of day and night. The temperature was maintained in the range of 19–25°C, relative humidity – 50–70%. The temperature and humidity were registered daily. Ventilation mode – 15 volumes of room air per hour. Animals were kept in standard cages (400x320x160 mm) in groups of 6. Diet – feed grains, bread, root vegetables (beets, carrots) [10,11]. Manipulation has been conducted according to the requirements of the use of animals in biomedical experiments [12,13].

Selected after the quarantine and preindividually marked animals by waterproof dye (Carbol fuchsin) were divided into the groups of 6 male rats in the absence of external signs of diseases and homogeneity groups by weight ($\pm 15\%$). Before the experiment each laboratory animal was weighted. Also, prior to the experiment, rats that have drowned or hung on the water surface were excluded from further manipulation.

The procedure of the experiment includes the following stages: first swimming until exhaustion; set of experimental groups according to the time of the first swimming by pairwise selec-

tion; second swimming until exhaustion, 5 min after the first; intragastric administration of the test compounds at a dose of 50 mg/kg; third swimming, 1 h after compound administration.

Swimming test was performed with the extra weight, which was attached at the base of the tail (10% of the rat body weight) in beforehand boiled water (to remove air bubbles). Temperature of 24–26°C (normothermia) and 10–12°C (hypothermia) was chosen for the experiment [14–16]. Swimming time was recorded until the signs of complete exhaustion that was revealed by rejection of further swimming, sinking to the bottom and unable to swim out for more than 10 sec. Rats were swimming separately in square size vessel, made of transparent glass (size 180x60x60 cm, at a height of 40 cm). The given methodology allowed to evaluate the physical performance of laboratory rats with mixed physical activity, which is implemented by aerobic-anaerobic system.

Intragastric administration of substances was performed *via* noninvasive probe in the form of an aqueous suspension with the addition of Tween-80, in a dose of 50 mg/kg, (Dose taken empirically). For control group equivalent volume of distilled water with Tween-80 was administered for animals. As reference drug, it is preferable to use «Bemithylum», however, this drug is not registered in Ukraine, that's why we have choose «Mildronate», as it was previously done in works of Kornienko [17,18]. The effectiveness of substances was compared with control group and reference drug «Mildronate» in similar experimental conditions. Dosage of «Mildronate» was taken the same (50 mg/kg), to have the results easier for comparison. The dynamics relative to control (DRC) was calculated by the following equation:

$$\text{DRC} = \frac{\text{Ave. swimming time (compd.)} - \text{Ave. swimming time (control)}}{\text{Ave. swimming time (control)}} \times 100$$

Statistical data processing was performed using a standard statistical package program, Version «MicrosoftOfficeExcel 2003», «STATISTICA® forWindows 6.0» (StatSoft Inc., № AXXR712D833214FAN5). Arithmetic mean (M) and standard error of the mean ($\pm m$) were calculated for each of the studied parameters. Data processing was performed by the method of variation statistics with definition Student's *t*-test, null hypothesis were declined if statistical criterion is $p < 0.05$.

Results and discussion

Considering the previously discovered actoprotective activity of 5-substituted *R*-thio-tetrazolo[1,5-*c*]quinazolines [2,3], it was decided to study a series of 6-substituted *N*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones, to explore the critical effect of substituents at the 5th or 6th position, and replacement of sulfur by oxygen. Thereby, to expand the library of tetrazolo[1,5-*c*]quinazolines with studied actoprotective activity.

It was established, that the intragastric administration of 6-*N*-*R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones (50 mg/kg) derivatives as well as «Mildronate» (50 mg/kg) was accompanied by physical endurance of animals in the stated conditions of the experiment, which evaluated their actoprotective activity. Thus, the duration of swimming increased for compounds **1**, **4** and **7** compared with control to 55.50%, 42.36% and 22.92% against



22.20% for «Mildronate», respectively (Table 1). However, compounds **5**, **6**, **2** and **3** revealed a slight decrease in duration of swimming, namely - 16.67%, -9.03%, -3.47% and -0.69%. It should be noted, that change of DRC of previously investigated 5-R-thio-tetrazolo[1,5-*c*]quinazolines was significantly higher.

Table 1

Effect of 6-*N*-R-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones and «Mildronate» at the duration of rat swimming in conditions of normothermia, *t* – 24–26°C, (*M*±*m*, *n*=6)

Compound, 50 mg/kg	Average swimming time, sec	Dynamics relative to control, %
Control	144±7	-
«Mildronate»	176±9 ^a	+22.20
1	224±20 ^a	+55.50
2	139±4 ^b	-3.47
3	143±4 ^b	-0.69
4	205±14 ^a	+42.36
5	120±9 ^{ab}	-16.67
6	131±5 ^b	-9.03
7	177±7 ^a	+22.92

Note: ^a – *p*≤0.05 in comparison to control; ^b – *p*≤0.05 in comparison to «Mildronate».

The most active compounds, namely, **1** and **4** were tested further in terms of hypothermia. Also like in terms of normothermia, DRC increased, at 20.24% for compound **1** and at 3.57% for compound **4**, against 7.14% for «Mildronate» (Table 2).

Therefore, it could be stated, that sulfur in the 5th position is a key element in exhibiting the actoprotective activity (taking into account known actoprotector, which had sulfur in its structure, «Bemithylum»). However, activity of 6-*N*-R-tetrazolo[1,5-

Table 2

Effect of 6-*N*-R-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones and «Mildronate» at the duration of rat swimming in conditions of hypothermia, *t* – 10–12°C, (*M*±*m*, *n*=6)

Compound, 50 mg/kg	Average swimming time, sec	Dynamics relative to control, %
Control	84±4	-
«Mildronate»	90±5	+7.14
1	101±3 ^a	+20.24
4	87±4	+3.57

Note: ^a – *p*≤0.05 in comparison to control.

c]quinazolin-5(6*H*)-ones hasn't disappeared too. The latter proved, that tetrazolo[1,5-*c*]quinazoline core itself had such properties. In that case, the introduction of the simplest methyl radical at the 6 position, led to increase of actoprotective activity of the investigated series.

Previously conducted studies have shown that quinazoline derivatives possess ergotropic action [19–21], which could be realized by increase of ATP production, at the expense of reactions normalization in the Krebs cycle. Thus, it can be assumed, that this ergotropic mechanism is a key in implementing of actoprotective activity.

Conclusions

1. Experimental studies on Wistar white rats revealed the actoprotective activity of 6-*N*-R-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones series. Rats swimming duration in conditions of normothermia and hypothermia had significantly increased. DRC increased for compound **1**, **4** and **7** to 55.50%, 42.36% and 22.92%, against 22.20% for «Mildronate».

2. Modifications to improve activity of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones will be conducted in the future.

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