THE EFFECTS OF NEW 1,3-THIAZOLIDINE-4-ONES WITH PYRAZOLONE SCAFFOLD ON MOTOR FUNCTION IN MICE

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Abstract

The aim of this study was to investigate the effects of new 1,3-thiazolidine-4-ones with pyrazolone scaffold (**7a-m**) on motor function in mice, using RotaRod performance and spontaneous locomotor activity tests. The toxicity screening of these compounds was also performed. The results of RotaRod performance test showed that the compounds don't have appreciable negative influence on neuromuscular activity, the recorded values being comparable with those of phenazone and control, so it could be appreciated that these compounds don't have neurotoxic effects. In the case of spontaneous locomotor activity test, the most intense negative effect was observed for **7j** (3-OH,4-OCH₃), for which the horizontal and vertical movements were lower than phenazone and control at all three-time intervals (1h, 2h, 4h). For other derivatives no appreciable negative effect on spontaneous locomotor activity was observed. Referring to the toxicity degree, all tested compounds showed lower toxicity in reference with phenazone, which supports the favourable influence of 1,3-thiazolidine-4-one moiety on pyrazolone scaffold.

Rezumat

Scopul acestui studiu a fost de a evalua efectele unor noi derivați de 1,3-tiazolidin-4-onă cu structură pirazolonică (**7a-m**) asupra funcției motorii la șoarece, utilizând testul tijei rotante (*RotaRod*) și testul activității locomotorii spontane. S-a determinat totodată și gradul de toxicitate al compușilor testați. Rezultatele testului tijei rotante au arătat că derivații studiați nu au o influență negativă apreciabilă asupra activității neuromusculare, valorile înregistrate fiind comparabile cu cele pentru fenazonă și lotul martor, putându-se astfel aprecia că derivații nu au efecte neurotoxice. În cazul activității locomotorii spontane, cel mai intens efect negativ a fost observat pentru compusul **7j** (3-OH,4-OCH₃), pentru care mișcările în plan orizontal și vertical au fost mai reduse comparativ cu fenazona și lotul martor, la toate cele trei intervale de timp studiate (1 oră, 2 ore, 4 ore). Pentru ceilalți derivați nu s-a observat un efect negativ apreciabil asupra activității locomotorii. Referitor la gradul de toxicitate, rezultatele au arătat că toți derivații studiați sunt mai puțin toxici comparativ cu fenazona, ceea ce susține influența favorabilă a ciclului de 1,3-tiazolidin-4-onă asupra structurii pirazolonice.

Keywords: 1,3-thiazolidin-4-one, pyrazolone scaffold, motor function, toxicity degree

Introduction

The pyrazolone scaffold is known mainly for analgesic, antipyretic and anti-inflammatory effects and some of its derivatives, such as phenazone, dipyrone, propyphenazone and aminophenazone are important therapeutic agents [1]. It is also known that the therapeutic use of these agents is often limited by their side effects, especially on haematological and gastrointestinal level. In order to improve the safety and effectiveness of pyrazolone derivatives, the researchers have focused their work to modulate this scaffold [1]. As a result, many pyrazolone derivatives have been developed and evaluated for other important effects such as antioxidant [2], antimicrobial [3] and anti-tumour [4].

On the other hand, the 1,3-thiazolidine-4-one ring gained much interest in the last years in the field of medicinal chemistry [5]. 1,3-thiazolidine-4-one derivatives were evaluated for antibacterial [5], anti-fungal [6, 7], antioxidant [8], cytotoxic [9], anti-inflammatory [10], antiviral [11] and anti-diabetic [12] effects.

Based on phenazone, our research group has synthesized new 1,3-thiazolidin-4-one derivatives whose synthesis was reported in our previous paper [13]. These derivatives showed improved antioxidant effects in reference with phenazone [13]. To continue our research, in this paper we present the evaluation results of these compounds for their effects on motor activity in mice using RotaRod test and assessment of spontaneous

locomotor activity. These tests are non-invasive tools and are usually used in preclinical evaluation of the drugs [14].

Materials and Methods

Materials

The study was performed using adult Swiss mice weighing 22 - 35 g (Cantacuzino Institute, Bucharest, Romania). The animals were housed at $23 \pm 2^\circ\text{C}$, humidity of 40 - 60%, with natural day-night cycle, having *ad libitum* access to food and water. The mice were kept fasting for 24h before the experiment

and were randomized into groups with 6 mice for each group. Thirteen new 1,3-thiazolidine-4-one derivatives based on pyrazolone scaffold (**7a-m**) were included in the study. The tested derivatives were synthesized starting from different substituted aromatic aldehydes (**1a-m**), ethyl 3-aminopropionate hydrochloride (**2**) and mercaptoacetic acid (**3**), when ethyl 3-(2-aryl-4-oxo-thiazolidin-3-yl)-propionate derivatives (**4a-m**) have been obtained. These intermediaries were transformed into corresponding acid derivatives (**5a-m**), which finally were coupled with 4-aminophenazone (**6**) [13] (Figure 1).

Figure 1.

The synthesis of 1,3-thiazolidine-4-one derivatives with pyrazolone scaffold (**7a-m**)

Acute toxicity assay

The toxicity degree of new 1,3-thiazolidine derivatives with pyrazolone scaffold (**7a-m**) was evaluated by determining of the lethal dose 50 (LD₅₀). The tested derivatives were orally administered (p.o.) using Tween 80 as vehicle in doses ranging between 500 - 3000 mg/kg bw. The survival rate was determined at 24h, 48h, 72h, 7 days and 14 days after administration. The lethal dose 50 (DL₅₀) was determined by Karber method using the following formula [14]:

$$LD_{50} = LD_{100} - \frac{\Sigma(a \times b)}{n},$$

where, a = the difference between two successive doses of the tested derivative; b = the arithmetic average of the animals from two successive series that died; n = the number of animals per group; LD₁₀₀ = the 100% lethal dose.

Motor function assays

The RotaRod performance test is one of the most used tests to assess the effect of drugs on animal behaviour, providing if the tested compounds have any effects on neuromuscular coordination [15]. In this test a commercially available RotaRod Apparatus (Ugo Basile Biological Research Apparatus, Italy) was used and the study was conducted according to general protocol [16]. The RotaRod consists of a circular rod turning at a constant or increasing speed. Animals placed on the rotating rod try to remain on it rather

than fall onto a platform. The drugs known to alter the neuromuscular coordination, such as the benzodiazepines, reduce the time in which the animals are able to remain on the rod. Only the mice with good locomotor activity which passed the RotaRod test, that means they were able to remain on the rod at least 60 sec, were included in the study. The effect of tested compounds (**7a-m**) on neuromuscular coordination and balance of the mice on the rod rotating with 15 rpm was evaluated during 180 sec.

Assessment of spontaneous locomotor activity. Spontaneous locomotor activity and exploratory behaviour of mice were monitored using the LE-8811 Actimeter (PanLAB Harvard Apparatus, Barcelona, Spain) [17, 18]. This equipment contains three plexiglass cages ($38 \times 24 \times 21$ cm internal dimensions), having around two-dimensional (horizontal and vertical axes) square frame connected to silent electronic counters. In the frames there are the infrared photo beams transmitters and receivers. During the test, horizontal locomotor activity (movements or stereotype movements) was determined by breaks in movement-sensitive photo beams that were then converted into locomotor activity counts, and vertical activity was recorded as the number of rearing episodes breaking the photocell beams of the upper frame. The frames are connected directly through a SeDaCom computer software, which allows easy exportation of data in a format compatible with Excel. Prior to the test, each animal was placed

in the centre of the arena, and the spontaneous locomotor behaviour was tracked for 5 min. The spontaneous locomotor activity was counted at 1 h, 2 h and 4 h after administration of the tested compounds. *Administration protocol of the tested compounds.* The 1,3-thiazolidine-4-one derivatives with pyrazolone scaffold (**7a-m**) were dissolved in Tween 80 to obtain

a stock solution of 10 mg/mL. From the stock solutions, each tested compound was administrated orally in dose of 1/20 LD₅₀, in a volume of 0.1 mL/10 g bw. Tween 80 (0.092 mL/10 g) and phenazone (56.50 mg/kg bw) were administrated to control and reference group respectively. The administered doses are presented in Table I.

Table I

The administrated doses (mg/kg bw) of tested compounds (**7a-m**)

Group	Compound/R	Dose (mg/kg bw)	Group	Compound/R	Dose (mg/kg bw)
1	7a/H	84.40	8	7h/2-NO ₂	86.00
2	7b/4-Cl	84.00	9	7i/3-NO ₂	83.50
3	7c/4-F	84.75	10	7j/3-OH,4-OCH ₃	87.50
4	7d/4-Br	86.00	11	7k/4-OH,3-OCH ₃	90.00
5	7e/2-OCH ₃	90.50	12	7l/4-CH ₃	84.00
6	7f/3-OCH ₃	87.50	13	7m/2,6-diCl	83.50
7	7g/4-OCH ₃	89.50	15	Phenazone (Phz)	56.50

The experiments were performed according to the ethics guidelines on laboratory animals studies (Law no. 206/May 27, 2004, EU/2010/63 – CE86/609/EEC) and with the agreement of the Ethic Committee for Animal Research of “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania.

Statistical analysis

The data were expressed as mean ± standard deviation. The analysis of data was performed using SPSS 17.0 software for Windows. In order to assure normal distribution of data, ANOVA one-way analysis of variance with Tukey's Post Hoc test were used for multiple comparisons. p values less than 0.05 were

considered statistically significant compared to control group.

Results and Discussion

Toxicity degree

The toxicological screening highlighted that all tested derivatives (**7a-m**) have low toxicity, the LD₅₀ values ranging between 1670 mg/kg bw (**7m**) and 1810 mg/kg bw (**7e**) (Table II). These compounds proved to be less toxic than phenazone (LD₅₀ = 1130 mg/kg bw) [19], which supports the favourable influence of thiazolidine-4-one moiety upon the chemical modulation of pyrazolone scaffold.

Table II

The toxicity degree (DL₅₀ values) of the tested compounds (**7a-m**)

Comp.	R	DL ₅₀ mg/kg bw)	Comp.	R	DL ₅₀ mg/kg bw)
7a	7H	1688	7h	2-NO ₂	1720
7b	4-Cl	1680	7i	3-NO ₂	1670
7c	4-F	1695	7j	3-OH,4-OCH ₃	1750
7d	4-Br	1720	7k	4-OH,3-OCH ₃	1800
7e	2-OCH ₃	1810	7l	4-CH ₃	1680
7f	3-OCH ₃	1750	7m	2,6-diCl	1670
7g	4-OCH ₃	1790	Phz	Phenazone	1130

The RotaRod performance test

For each tested compound (**7a-m**), it was recorded the time (sec) in which the mice remained on the rotating rod. For the most compounds, the obtained values were comparable with those recorded for phenazone and control (Figure 2). A slight negative influence was observed for compounds **7j** (3-OH, 4-OCH₃), **7k** (3-OCH₃, 4-OH), **7d** (4-Br) and **7f** (3-OCH₃). For these compounds the values recorded at 4 h after administration were 117.6 ± 19.3 (**7j**), 120.0 ± 16.2 (**7k**), 121.7 ± 21.7 (**7d**), 129.8 ± 17.7

(**7f**), this being slightly lower than phenazone (158.0 ± 24.6) and control (142.0 ± 19.6) respectively. We can appreciate that the tested derivatives (**7a-m**), as well as phenazone, have not appreciable influence on neuromuscular activity in mice, because it was not observed the loss of equilibrium which could be associated with neurotoxic effects. No statistically significant differences (p > 0.05) between the tested compounds (**7a-m**) and phenazone and control, at all time intervals (1 h, 2 h, 4 h), were observed.

Figure 2.

The RotaRod test results recorded for the tested compounds (**7a-m**) in reference with phenazone (Phz) and control (Tween 80) groups

Assessment of spontaneous locomotor activity

The changes of mice behaviour were recorded as horizontal (licking of paw, cleaning of hair and of nose) and vertical movements (climbing and jumping on walls of cage), and could be associated with anxiety or depressive-like state. The results of mice behaviour at 1 h, 2 h and 4 h after the administration of tested derivatives (**7a-m**), in reference with phenazone and control are presented in Figure 3. and Figure 4.

Referring to the horizontal movements, the most intense negative effect was observed for **7j** (3-OH,4-OCH₃), for which the recorded values were lower than Phenazone and Control at all three time intervals (1h, 2h, 4h), the results being statistically

significant ($p < 0.05$) in reference with Control. For example, at 4h after administration, the value recorded for **7j** was 162.8 ± 13.8 while the values recorded for phenazone and control were 298.6 ± 24.8 and 363.0 ± 26.1 respectively. A slightly negative effect was observed also, at 1h after administration, for **7e** (2-OCH₃, 299.6 ± 21.4), **7f** (3-OCH₃, 253.8 ± 23.6), **7i** (3-NO₂, 271 ± 22.3), **7l** (4-CH₃, 272.5 ± 21.8), but the results are not statistically significant in reference with control (Figure 3). In similar conditions for **7m** (2,6-diCl) it was observed a positive effect, the recorded horizontal movements being higher than phenazone and control.

Figure 3.

The horizontal movements recorded for the tested compounds (**7a-m**) in reference with phenazone (Phz) and control (Tween 80) groups (• $p < 0.05$ in reference with control)

Referring to the vertical movements the most negative effect was observed for **7h** (2-NO₂, 0.6 ± 0.4) and **7l** (4-CH₃, 1.00 ± 0.4), the values recorded at 1 h after administration being 10 times and 6 times, respectively lower in reference with control (6.1 ± 2.5) ($p < 0.001$) (Figure 4). At 4 h after administration, an intense negative effect was observed also for **7j** (3-OH,

4-OCH₃, 1.4 ± 0.7 , $p < 0.05$), **7c** (4-F, 2.0 ± 0.9 , $p < 0.001$), **7b** (4-Cl, 2.0 ± 0.9 , $p < 0.001$), **7k** (4-OH, 3-OCH₃, 2.2 ± 1.1 , $p < 0.05$). For **7a** (H), **7d** (4-Br), **7g** (4-OCH₃) and **7m** (2,6-diCl), as for Phenazone, no negative influence on vertical movements was observed.

Figure 4.

The vertical movements recorded for the tested compounds (7a-m) in reference with phenazone (Phz) and control (Tween 80) groups (+ $p < 0.001$, ♦ $p < 0.05$, in reference with control)

Conclusions

The toxicity degree and the effects on motor function in mice, using RotaRod performance test and assessment of spontaneous locomotor activity, of new thirteen 1,3-thiazolidine-4-ones with pyrazolone (7a-m), scaffold was evaluated. The results showed the lower toxicity of tested compounds in reference with phenazone, which supports the favourable influence of 1,3-thiazolidine-4-one moiety on pyrazolone scaffold. Based on the RotaRod performance test results it could be appreciated that the most of the tested compounds don't have neurotoxic effects because no appreciable negative influence on neuromuscular activity in mice was observed. This is also supported by the results of spontaneous locomotor activity test, counted as horizontal vertical movements of mice, when no negative effects were observed for most part of the tested compounds.

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