Diuretic activity of some 4- and 3-thio-substituted 1,2,4-triazoles with fluorophenyl fragments

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Diuretics increase the volume of urine and sodium excretion, and therefore are often used to remove excess fluid from the body during various diseases, including hypertension, heart and kidney failure, nephrotic syndrome, cirrhosis, etc. Until now, a wide range of diuretics is known, but none of them is completely safe and has a number of side effects.

The aim of the work is to investigate the diuretic activity of 1,2,4-triazole derivatives containing fluorophenyl substituents at the fifth position of the 1,2,4-triazole fragment, as well as to establish the relationship between “chemical structure and diuretic action” among derivatives 1,2,4-triazole.

Materials and methods. To study the effect on kidney function, six groups of animals with seven rats each were used. When studying water diuresis, rats were kept on a constant diet with free access to water. Before water loading (5 % of body weight), rats were fasted for 2 hours without food and water. Since all tested compounds were insoluble in water, they were administered orally as an aqueous suspension stabilized with Tween-80. The compounds were administered at a dose of 1/10 LD50.

Results. The diuretic activity of 28 new compounds of a number of substituted 1,2,4-triazole-3-thiols, which according to their chemical structure belong to different classes of compounds, was investigated.

Conclusions. Analysis of the results of diuretic activity indicates that there are a number of compounds whose activity is higher than hypotiazide, as well as a compound that exceeds the activity of furosemide.

Key words: diuretic effect, 1,2,4-triazole derivatives, “structure – action”.

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The achievements of Ukrainian scientists in the field of synthetic organic chemistry significantly contribute to the development of world science. Among the variety of known organic compounds, the heterocyclic system of 1,2,4-triazole deserves special attention, as it has been at the center of research for decades [1,2,5]. The 1,2,4-triazole cycle has unique properties, which make it a very attractive target for research [3,4].

Numerous publications by various scientific teams from around the world have demonstrated the high reactivity of 1,2,4-triazole derivatives, as well as their low toxicity [6,10], and a wide range of possibilities for their use as potential biologically active compounds [7–9]. Promising properties of some 1,2,4-triazole derivatives, which are used as effective plant growth regulators, have been described in [8,11]. It is worth emphasizing the possibility of using 1,2,4-triazole derivatives as veterinary drugs [9,10].

In particular, the drug “Trifuzol-Neo” is widely used in veterinary medicine, which has recently gained popularity due to its effectiveness. At the stage of registration is a new original antifungal veterinary drug in the form of liniment “VetMicoDerm”. Therefore, the continuation of research related to the synthesis of new derivatives of 1,2,4-triazole, as well as the study of the pharmacological properties of new derivatives is an important scientifically attractive area, which undoubtedly offers promising opportunities in various aspects of human life.

In our opinion, the study of the diuretic activity of new 1,2,4-triazole derivatives deserves special attention. First, the creation of new diuretics is relevant today, and secondly, there is information that some 1,2,4-triazole derivatives have high diuretic activity [9]. Therefore, the aim of our work was to investigate the diuretic activity of new substituted 1,2,4-triazole-3-thiols.

**Aim**

The aim of the work is to study the diuretic activity of 1,2,4-triazole derivatives containing fluorophenyl substituents at the fifth position of the 1,2,4-triazole fragment, as well as to establish the relationship between “chemical structure and diuretic action” among derivatives 1,2,4-triazole.

**Materials and methods**

In our research, we used a series of new substituted 1,2,4-triazole-3-thiols (Fig. 1), which were first synthesized in the laboratory of organic synthesis of the Department of Natural Sciences for foreign students and toxicological chemistry of Zaporizhzhia State Medical and Pharmaceutical University.

Physical-chemical constants of the synthesized compounds have been studied and described before, some of them are given in the literature [1,10].

Previously, we conducted a study to determine the dose of synthesized compounds, we also studied the general toxic effect and acute toxicity of these derivatives. The research was carried out according to the well-known express method of V. B. Prozorovsky, and the assessment of the degree of toxicity was determined by K. K. Sidorov. The animals were kept on a standard diet, under natural light regime “day and night” [12]. Further studies were performed taking into account the “Rules of preclinical safety assessment of pharmacological agents (GLP)” [13]. Acute toxicity studies of each compound were studied in a range of four doses, each of which was tested on 2 animals [14,16].

The follow-up period was 14 days, during which the nature and duration of symptoms of intoxication, the time of death, and the number of dead animals from each dose were taken into account [15,16]. The research was conducted in accordance with the national “General Ethical Principles of Animal Experiments” (Ukraine, 2001), which is consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1985).

To study the effect on renal function, 6 groups of animals with 7 rats in each were used. In the study of aqueous diuresis, rats were kept on a constant diet with free access to water. Prior to water loading (5 % of body weight), rats were kept for 2 h without food and water. All compounds studied were water-insoluble, so they were administered orally as an aqueous suspension stabilized with tween-80. The compounds were administered at a dose of 1/10 of LD<sub>50</sub>.

The amount of urine was measured every hour for 4 hours. The amount of urine excreted by the control group of animals (which did not receive test compounds) was taken as 100 %. The study and analysis of the obtained experimental data were performed in comparison with the reference diuretics: hypothiazide and furosemide.

**Results**

Twenty-eight new compounds, including substituted 1,2,4-triazole-3-thiols belonging to different classes of compounds based on their chemical structure, were studied for their diuretic activity (Fig. 1).

Among the 3-(2-,3-fluorophenyl)-6-(aryl-,heteryl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazoles, four exceed the reference diuretic hypothiazide (2, 4, 5, 6, Table 1), and 3-(2-fluorophenyl)-6-(phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (1), 3-(4-fluoro phenyl)-6-(3-methoxyphenyl)-[1,2,4] triazole[3,4-b][1,3,4]thiadiazole (7) and 3-(3-fluorophenyl)-6-(2-bromo-4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (8) approximate by activity to furosemide. Regarding the activity of 3-(2,3-fluorophenyl)-6-R<sub>n</sub> -7H[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (9–14, Table 1), high rates of diuretic action were not detected.

**Discussion**

Analysis of the results of the diuretic activity of 3-alkylthio-5-(3-fluorophenyl)-4-methyl-1,2,4-triazoles (15–18, Table 1) shows a fairly high activity against hypothiazide, especially for 3-propylthio-5-(3-fluorophenyl)-4-methyl-1,2,4-triazole (15) and 3-hexylthio-5-(3-fluorophenyl)-4-methyl-1,2,4-triazole (18).
Table 1. The results of the study of the diuretic activity of some substituted 1,2,4-triazole-3-thiols

<table>
<thead>
<tr>
<th>No. connection</th>
<th>120 min, M ± m, ml</th>
<th>∆ % to control</th>
<th>240 min, M ± m, ml</th>
<th>∆ % to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.330 ± 0.113</td>
<td>—</td>
<td>2.130 ± 0.117</td>
<td>—</td>
</tr>
<tr>
<td>Furosemide</td>
<td>4.0800 ± 0.0352*</td>
<td>186.07</td>
<td>6.050 ± 0.028*</td>
<td>188.77</td>
</tr>
<tr>
<td>Hypothiazide</td>
<td>2.710 ± 0.332*</td>
<td>83.12</td>
<td>3.610 ± 0.312*</td>
<td>77.18</td>
</tr>
<tr>
<td>1</td>
<td>3.660 ± 0.438*</td>
<td>96.08</td>
<td>6.110 ± 0.366*</td>
<td>137.18</td>
</tr>
<tr>
<td>2</td>
<td>2.770 ± 0.074*</td>
<td>72.54</td>
<td>5.590 ± 0.310*</td>
<td>94.33</td>
</tr>
<tr>
<td>3</td>
<td>2.090 ± 0.166</td>
<td>33.45</td>
<td>3.210 ± 0.208*</td>
<td>32.32</td>
</tr>
<tr>
<td>4</td>
<td>2.230 ± 0.484</td>
<td>54.17</td>
<td>4.670 ± 0.458*</td>
<td>96.28</td>
</tr>
<tr>
<td>Control</td>
<td>1.630 ± 0.159</td>
<td>—</td>
<td>2.740 ± 0.265</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>2.840 ± 0.088*</td>
<td>72.44</td>
<td>5.780 ± 0.209*</td>
<td>94.18</td>
</tr>
<tr>
<td>6</td>
<td>2.720 ± 0.187</td>
<td>73.22</td>
<td>5.750 ± 0.187</td>
<td>95.11</td>
</tr>
<tr>
<td>7</td>
<td>3.180 ± 0.344</td>
<td>96.09</td>
<td>6.170 ± 0.456</td>
<td>138.19</td>
</tr>
<tr>
<td>8</td>
<td>3.150 ± 0.445</td>
<td>96.12</td>
<td>6.130 ± 0.413</td>
<td>139.32</td>
</tr>
<tr>
<td>9</td>
<td>2.120 ± 0.222</td>
<td>17.17</td>
<td>3.280 ± 0.216</td>
<td>63.16</td>
</tr>
<tr>
<td>Control</td>
<td>1.330 ± 0.088</td>
<td>—</td>
<td>2.210 ± 0.249</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>2.130 ± 0.212*</td>
<td>43.77</td>
<td>4.210 ± 0.260*</td>
<td>73.13</td>
</tr>
<tr>
<td>11</td>
<td>1.540 ± 0.133*</td>
<td>3.35</td>
<td>3.110 ± 0.228</td>
<td>17.74</td>
</tr>
<tr>
<td>12</td>
<td>1.570 ± 0.214</td>
<td>15.54</td>
<td>2.420 ± 0.115</td>
<td>40.65</td>
</tr>
<tr>
<td>13</td>
<td>1.870 ± 0.175</td>
<td>33.54</td>
<td>3.300 ± 0.211*</td>
<td>31.55</td>
</tr>
<tr>
<td>14</td>
<td>3.240 ± 0.155</td>
<td>30.07</td>
<td>5.180 ± 0.319</td>
<td>69.21</td>
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<tr>
<td>15</td>
<td>4.110 ± 0.113</td>
<td>160.23</td>
<td>7.560 ± 0.221</td>
<td>162.16</td>
</tr>
<tr>
<td>Control</td>
<td>1.350 ± 0.172</td>
<td>—</td>
<td>2.520 ± 0.115</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>2.380 ± 0.609</td>
<td>53.85</td>
<td>4.750 ± 0.563</td>
<td>95.62</td>
</tr>
</tbody>
</table>

Fig. 1. Structure of some substituted 1,2,4-triazole-3-thiols.
Molecules of 5-(2-,3-fluorophenyl)-4-(aryl-,heteryl)ylidene)amino-1,2,4-triazole-3-thiols (19–24, Fig. 1) consist of various functional substituents. Therefore, it is possible to assume with a certain probability that their activity depends on features of structure of substituents on amino group.

Thus, the introduction of a 3-fluorophenyl or 4-fluorophenyl substituent into the molecule leads to an increase in diuretic activity almost twice relative to hypothiazide (21, 22, Table 1). Of particular note is 5-(3-fluorophenyl)-4-(4-fluorophenylamino)-1,2,4-triazole-3-thiol (25, Table 1), whose diuretic activity is highest.

The obtained data were statistically processed using the standard software package Microsoft Office 2007 and Statistica for Windows 6.0. The significance of intergroup differences according to experimental data was established using Student’s t-test. The level of statistical significance of differences in research results is $p < 0.01$. Analyzing the results of the experiment, it was found that some compounds that were studied in a number of new derivatives of 1,2,4-triazole-3-thiol, have a pronounced diuretic activity (Table 1).

### Conclusions

1. The diuretic activity of a number of new 4- and 3-thio-substituted 1,2,4-triazoles with fluorophenyl fragments was studied for the first time.

2. A thorough analysis of the results of diuretic activity reveals several compounds with higher activity than hypothiazide, as well as a compound with greater activity than furosemide.

3. In some cases, certain patterns between the chemical structure of the molecules and their diuretic activity were observed.

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#### References


[3] Parchenko, V. V. (2006). Syntez, fizyko-khimichni ta biolohichni vlastivosti pokhidnykh 1,2,4-triazol-3-thionu, yaki mistiat yadro furanu [Synthesis, physical, chemical, and biological properties of 1,2,4-triazol-3-thione derivatives, which contain furane cycle] (PhD dissertation). Zaporizhzhia State Medical University, Zaporizhzhia. [in Ukrainian].


[9] Parchenko, V. V. (2014). Syntez, peretvorennia, fizyko-khimichni ta biolohichni vlastivosti v riadi 5-furylzamishchenykh 1,2,4-triazol-3-thioniv (Dis. dokt. farm. nauk) [Synthesis, transformation, physico-chemical and biological properties in the number of 5-furylsubstituted 1,2,4-triazole-3-thiones (Doctoral dissertation)]. Zaporizhzhia State Medical University, Zaporizhzhia. [in Ukrainian].


