Antiradical activity of novel 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives

A. A. Safonov\textsuperscript{A,B,C,D}, I. S. Nosulenko\textsuperscript{A,C,D}

Zaporizhzhia State Medical University, Ukraine

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

The process of studying free radicals began in the middle of the last century (the free radical theory of aging in 1956). Multiple studies have revealed the effect of free radicals on the cells of the body and the development of various diseases, such as diabetes, autoimmune diseases, diseases of the nervous system, and others. As a result, the term antioxidant has emerged, compounds that reduce and prevent the effects of free radicals. Most of the newly synthesized substances are studied for their antiradical properties. 1,2,4-Triazole derivatives are no exception, which have already proven themselves as biologically active compounds.

The aim of this work was the investigation antiradical activity among 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives.

Materials and methods. Previously synthesized 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives were used as test compounds. The research of antiradical activity was based on the interaction between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro. DPPH is a stable free radical. The color of its alcoholic solutions were intense purple ($\lambda_{\text{max}} = 517$ nm). When DPPH interacted with compounds that were capable of scavenging free radicals, it produced products. These products are yellow in color and do not absorb light of the aforementioned wavelength. The study was carried out according to the method.

Results. The antiradical activity of 10 new 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives was studied. Most of the test compounds show antiradical activity against DPPH. Compound 1 was the most active at a concentration of $1 \times 10^{-3}$ M and the antiradical effect was close to ascorbic acid.

Conclusions. The most active compound is 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol, which in a concentration of $1 \times 10^{-3}$ M has an antiradical effect in 88.89 %. When reducing the concentration to $1 \times 10^{-4}$ M, also reduces the antiradical activity to 53.78 %. Some conclusions are drawn regarding the “structure – effect” dependence between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives:

– the introduction of 4-fluorobenzylidene radical (compound 2) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule results in a slight decrease in activity;

– the introduction of 2-hydroxybenzylidene radical (compound 3) into initial molecule results a high antiradical effect, which hardly changes with decreasing concentration;

– transformation to 2-((5-(thiophen-2-ylmethyl)-4-((R)amino)-4H-1,2,4-triazol-3-yl)thio)acetic acid has almost no effect on antiradical activity, except for compound 9 (the antiradical effect is reduced).

Key words: antiradical activity, triazoles, heterocyclic compounds.

Antирадикальна активність нових похідних 4-аміно-5-(тіофен-2-ілметил)-4Н-1,2,4-триазол-3-тиолу

А. А. Сафонов, І. С. Носуленко

Вивчення вільних радикалів розпочалося в середині минулого сторіччя (вільнорадикальна теорія старіння, 1956 р.). Численні дослідження виявили вплив вільних радикалів на клітини організму та розвиток у результаті цього різних захворювань: цукрового діабету, автоімунних захворювань, патологій нервової системи тощо. У зв’язку з цим з’явилася такий термін, як антиоксиданти, на позначення сполук, що зменшують і зобов’язують вплив вільних радикалів. Більшість нових синтезованих речовин досліджують на наявність антирадикальних властивостей. Не є винятком похідні 1,2,4-триазолу, що зарекомендували себе як біологічно активні сполуки.

Мета роботи – дослідження антирадикальної активності серед похідних 4-аміно-5-(тіофен-2-ілметил)-4Н-1,2,4-триазол-3-тиолу.

Current issues in pharmacy and medicine: science and practice 2021; 14 (2), 162–166

UDC 615.281/282.015/615.27.015.11
DOI: 10.14739/2409-2932.2021.2.230129

Key words: antiradical activity, triazoles, heterocyclic compounds.

*E-mail: 8safonov@gmail.com

Received: 29.04.2021 // Revised: 06.05.2021 // Accepted: 11.05.2021
Антирадикальна активність нових приготуваних 4-аміно-5-(тиофен-2-илметил)-1,2,4-триазол-3-тиола

А. А. Сафонов, І. С. Носуленко

Процес ізування свободних радикалів почався ще в середині прошлого століття (свободнорадикальна теорія старіння, 1956 р.). Многочисленні дослідження показали вплив свободних радикалів на клетки організма і розвиток в реаліті цього різних хвороб: сахарного диабета, аутоімунних захворювань, захворювань нервової системи і т.д. У відповідь до цього був уведені такі терміни, як антиоксиданти, для обозначення сполук, які зменшують і відхилюють вплив свободних радикалів. Большинство нових синтезованих сполук є засадово активними сполуками.

Матеріал та методи. Дослідження були здійснені в рамках проекту по розвитку антирадикальної активності у радикалах 4-аміно-5-(тиофен-2-илметил)-4H-1,2,4-триазол-3-тиолу. Дослідження антирадикальної активності зоснаване на взаємодії радикалів 4-аміно-5-(тиофен-2-илметил)-4H-1,2,4-триазол-3-тиолу з 2,2-дифенил-1-пікринол (DPPH) in vitro. DPPH – стабільний вільний радикал. Колір його спиртових розчинів — інтенсивно-фіолетовий (λmax = 517 нм). Колір DPPH взаємодіє зі сполуками, що здатні впливати на анітуди радикали, він утворює комплекси. Ці сполуки мають жовтий колір та не поглинають світло з названою довжиною хвилі. Дослідження виконані згідно з методикою.

Результати. Визначено антирадикальну активність 10 нових сполук похідних 4-аміно-5-(тиофен-2-илметил)-4H-1,2,4-триазол-3-тиолу. Більшість сполук виявили антирадикальну активність щодо DPPH. Речовина 1 найбільш активна в концентрації 1 × 10^{-3}, а нова антирадикальна активність близько до аскорбінової кислоти.

Висновки. Найактивніша сполука – 4-аміно-5-(тиофен-2-илметил)-4H-1,2,4-триазол-3-тиол, який у концентрації 1 × 10^{-4} M характеризується антирадикальною дією в 88,89 %. При зниженні концентрації до 1 × 10^{-4} M антирадикальна активність знижується до 53,78 %. Зроблено висновки щодо залежності структура — антирадикальна дія.

Ключові слова: антирадикальна активності, 1,2,4-триазол, гетероциклічні сполуки.


Antiradical activity of novel 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives

A. A. Safonov, I. S. Nosulenko

The process of studying free radicals began in the middle of the last century (the free radical theory of aging in 1956). Multiple studies have revealed the effect of free radicals on the cells of the body and the development of various diseases, such as diabetes, autoimmune diseases, diseases of the nervous system, and others. The study of the effects of free radicals on living organisms is a very interesting topic for discussion. Most scientists and
doctors believe that in order to prolong the life of cells, it is necessary to reduce the number of free radicals in the human body. In this case, special compounds can help. As a result, the term antioxidant has emerged, compounds that reduce and prevent the effects of free radicals. Most of the newly synthesized substances are studied for their antiradical properties [1–3]. 1,2,4-Triazole derivatives are no exception [4], which have already proven themselves as biologically active compounds [5–10].

**Aim**

The aim of this work was to investigate antiradical activity among 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives.

**Materials and methods**

4-Amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives which previously synthesized were used as test compounds [11,12].

The research of antiradical activity was based on the interaction between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro. DPPH is a stable free radical. The color of its alcoholic solutions is intense purple (λmax = 517 nm). When DPPH interacts with compounds that are capable of scavenging free radicals, it produces products. These substances are yellow in color and do not absorb light of the aforementioned wavelength. The study was carried out according to the method [13,14].

**Research methodology.** 2 ml of solution of 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives in DMSO of corresponding concentration (2 mM, 0.2 mM, 0.01 mM, 0.02 mM, 0.002 mM) was mixed with 2 ml of 0.1 mM DPPH methanolic solution. Optical density (Ad) was measured after incubating the resulting mixture at 25 °C for 30 minutes. The sample was obtained by mixing 2 ml of DMSO with 2 ml of 0.1 mM methanolic DPPH solution and determined the optical density simultaneously (ADPH).

Antiradical activity (ARA) was calculated by the next formula:

\[ \text{ARA\%} = \frac{(A_{\text{ADPH}} - \text{Ad})}{A_{\text{ADPH}}} \times 100\% \]

In the case of a negative meaning, ARA in % was estimated at 0. Electronic balance “ANG 200C” (Poland) was used to weigh reagents and synthesized compounds. Optical density was measured with a ULAB 108UV spectrophotometer (China).

**Results**

The antiradical activity of 10 new 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives was studied. Most of the test compounds showed antiradical activity against DPPH. Compound 1 was the most active at a concentration of 1 × 10⁻³ M and the antiradical effect was close to ascorbic acid (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antiradical activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>92.23</td>
</tr>
<tr>
<td>1</td>
<td>88.89</td>
</tr>
<tr>
<td>2</td>
<td>78.26</td>
</tr>
<tr>
<td>3</td>
<td>73.91</td>
</tr>
<tr>
<td>4</td>
<td>75.68</td>
</tr>
<tr>
<td>5</td>
<td>74.24</td>
</tr>
<tr>
<td>6</td>
<td>72.46</td>
</tr>
<tr>
<td>7</td>
<td>78.26</td>
</tr>
<tr>
<td>8</td>
<td>78.42</td>
</tr>
<tr>
<td>9</td>
<td>10.31</td>
</tr>
<tr>
<td>10</td>
<td>3.06</td>
</tr>
</tbody>
</table>

According to the results of the investigation, “structure – effect” dependence between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives was researched.

The most active compounds were substances 3 and 8 at a concentration of 1 × 10⁻⁴ M. Thus, the best effect of (2-hydroxybenzylidene)amino and (thiophen-2-ylmethylene) amino radicals on antiradical activity.

**Discussion**

The antiradical effect of 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives was studied and “structure – effect” dependence was established. The most active compound was 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol, which in a concentration of 1 × 10⁻³ M had an antiradical effect in 88.89 %. When the concentration was reduced to 1 × 10⁻⁴ M, the antiradical activity was reduced to 53.78 %. (Table 2).

The adding of 4-fluorobenzylidene radical (compound 2) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule was resulted in a slight decrease in activity (Fig. 1). Dilution of the solution to 1 × 10⁻⁴ M also was reduced the antiradical effect (Fig 2).

The same pattern was observed with the adding of thiophen-2-ylmethylene (compound 4) and 1-(4-aminophenyl) ethylidene (compound 5) radicals into the molecule 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol. When the 2-hydroxybenzylidene radical (compound 3) was added into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule, a high antiradical effect was observed, which hardly changes with decreasing concentration. A similar dependence was shown by compound 8 (2-((5-(thiophen-2-ylmethyl))-4-(thiophen-2-ylmethylene) amino)-4H-1,2,4-triazol-3-yl)thioacetic acid), which showed an antiradical effect of 78.42 % (1 × 10⁻³ M) and 78.10 % (1 × 10⁻⁴ M) at appropriate concentrations.
Transformation to 2-((5-(thiophen-2-ylmethyl)-4-amino-4H-1,2,4-triazol-3-yl)thio)acetic acid had almost no effect on antiradical activity, except for compound 9. In this case, the antiradical activity was significantly reduced. Transformation to 2-((5-(thiophen-2-ylmethyl)-4-amino-4H-1,2,4-triazol-3-yl)thio)acetic acid salts reduced antiradical activity, which can be explained by blocking the carboxyl group.

**Conclusions**

1. For 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives antiradical activity with stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro was investigated. The most active compound was 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol, which in a concentration of 1 × 10⁻³ M had an antiradical effect in 88.89 %. When reducing the concentration to 1 × 10⁻⁴ M, also reduced the antiradical activity to 53.78 %.

2. Some conclusions were drawn regarding the "structure – effect" dependence between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives:
   - the adding to 4-fluorobenzylidene radical (compound 2) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 4-hydroxybenzylidene radical (compound 3) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 1-(4-aminophenyl)ethylidene radical (compound 5) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 4-aminophenyl radical (compound 9) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 4-fluorobenzylidene radical (compound 6) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 4-hydroxybenzylidene radical (compound 7) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;
   - the adding to 1-(4-aminophenyl)ethylidene radical (compound 8) into the 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol molecule resulted in a slight decrease in activity;

**Fig 1.** The antiradical activity of 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives (1 × 10⁻³ M).

**Fig 2.** The antiradical activity of 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives (1 × 10⁻⁴ M).

Table 2. "Structure – activity" dependence between 4-amino-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol derivatives.

<table>
<thead>
<tr>
<th>Substance</th>
<th>R</th>
<th>R₁</th>
<th>Anti-radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>NH₂</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>(4-fluorobenzylidene)amin</td>
<td>↑</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>(2-hydroxybenzylidene)amin</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>(thiophen-2-ylmethylene)amin</td>
<td>↑</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>(1-(4-aminophenyl)ethylidene)amin</td>
<td>↑</td>
</tr>
<tr>
<td>6</td>
<td>CH₂COOH</td>
<td>(4-fluorobenzylidene)amin</td>
<td>↑</td>
</tr>
<tr>
<td>7</td>
<td>CH₂COOH</td>
<td>(2-hydroxybenzylidene)amin</td>
<td>↑</td>
</tr>
<tr>
<td>8</td>
<td>CH₂COOH</td>
<td>(thiophen-2-ylmethylene)amin</td>
<td>↑↑</td>
</tr>
<tr>
<td>9</td>
<td>CH₂COOH</td>
<td>(1-(4-aminophenyl)ethylidene)amin</td>
<td>↓</td>
</tr>
<tr>
<td>10</td>
<td>CH₂COONa</td>
<td>NH₂</td>
<td>↓</td>
</tr>
</tbody>
</table>
the adding to 2-hydroxybenzylidene radical (compound 3) into initial molecule resulted a high antiradical effect, which hardly changed with decreasing concentration; transformation to 2-((5-(thiophen-2-ylmethyl)-4-((R) amino)-4H-1,2,4-triazole-3-yl)thio)acetic acid had almost no effect on antiradical activity, except for compound 9 (the antiradical effect is reduce).

Funding
The research is carried out within the SRW of Zaporizhzhia State Medical University "Synthesis, modification and study of the properties of 1,2,4-triazole derivatives in order to create an antimicrobial drug", state registration No. 0120U101649.

Conflicts of interest: authors have no conflict of interest to declare.

Information about authors:
Safonov A. A., PhD, Associate Professor of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0003-2861-1826
Nosulenko I. S., PhD, Senior Lecturer of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-8725-7321

Vідомості про авторів:
Сафонов А. А., канд. фарм. наук, доцент каф. фармакогнозії, фармакології та ботаніки, Запорізький державний медичний університет, Україна.

Сведения об авторах:
Сафонов А. А., канд. фарм. наук, доцент каф. фармакогнозии, фармакологии и ботаники, Запорожский государственный медицинский университет, Украина.

References
[1] Kaplaushenko, A. H. (2013). Doslidzhennia zna storevienia novoho oryhnalino vitychnoznanno liksarsko zo zasob na osnovi 1,2,4-триазолu [The research of creating a new original domestic drug based on 1,2,4-triazole]. Naukovy zhurnal MOZ Ukrainy, 2(3), 115-121. [in Ukrainian].
[5] Shcherbyna, R. O., Kapelyanoych, Ye. V., Pruho, Ye. S., Panasenko, O. I., & Knys, Ye. H. (2014). Doslidzhennia aktoprotektornoi aktynosti pokhidnykh 4-R-3-(morfolinometyleny)-1,2,4-triazol-5-tiolu [The studying of actoprotective action of 4-R-3-(morpholinomethylene)-1,2,4-triazole-5-thio derivatives]. Odeskiy medychnyi zhurnal, (6), 19-22. [in Ukrainian].
[9] Samelyuk, Yu. H., & Kaplaushenko, A. H. (2015). Hostra toksychnistъ 5-(2-, 3-, 4-metoksifenil, (3,4,5-trymetoksyfenil)-1,2,4-triazol-3-tioniv ta yikh tiopokhidnykhy [Acute toxicity of 5-(2-, 3-, 4-methoxyphenyl, (3,4,5-trimethoxyphenyl)-1,2,4-triazole-3-thiones and their thioderivatives]. Current issues in pharmacy and medicine: science and practice, (3), 57-60. [in Ukrainian]. https://doi.org/10.14739/2409-2932.2015.3.52660