Evaluation of antioxidant activity of 1,2,4-triazole derivatives in the initiation of free radical processes

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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

The activation of lipid free radical oxidation is a key stage in the development of many diseases and can lead to the emergence of related complications. To identify new synthetic compounds with antioxidant properties, it is necessary to conduct studies on various models of experimental free radical oxidation of biomolecules. Literature sources indicate that among the derivatives of 1,2,4-triazole, there is a significant number that possess high antioxidant activity.

The aim of the study to assess the antioxidant properties of newly synthesized S-derivatives of 1,2,4-triazole through the inhibition of reactive oxygen species accumulation in the superoxide dismutase system.

Materials and methods. To achieve this goal, we used one of the methods for assessing antioxidant activity by initiating free radical processes in vitro, specifically by inhibiting the accumulation of reactive oxygen species.

Results. Twenty-three S-derivatives of 5-(thiophene-3-ylmethyl)-4-R-1,2,4-triazole-3-thiols were investigated. The most effective compound was sodium 2-((4-phenyl-5-thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)acetate, which exceeded the activity of both reference drugs. Following in activity was compound 22, 1-phenyl-2-((4-phenyl-5-(thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)ethanol, which demonstrated an effect similar to that of emoxipine, while compound 7, 1-(3-fluorophenyl)-2-((5-thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)ethanone, was slightly less active.

Conclusions. It was established that among the new S-derivatives of 1,2,4-triazole, some exhibit significant antioxidant effects that are comparable to or exceed the efficacy of reference drugs.

Keywords: 1,2,4-triazole, antioxidant activity, superoxide dismutase.

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Оцінювання антиоксидантної активності похідних 1,2,4-тріазолу при ініціюванні вільнорадикальних процесів

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Активація вільнорадикального окиснення ліпідів є ключовим етапом у розвитку багатьох захворювань та може буди причиною виникнення супутніх ускладнень. Для виявлення нових синтетичних сполук з антиоксидантними властивостями необхідно їх досліджувати на різних моделях експериментального вільнорадикального окиснення біомолекул. Відомості наукової літератури свідчать, що чимало похідних 1,2,4-тріазолу мають високу антиоксидантну активність.

Мета роботи – оцінити антиоксидантні властивості нових синтезованих S-похідних 1,2,4-тріазолу через пригнічення накопичення активних форм кисню в системі супероксиддисмутази.

Матеріали і методи. Для досягнення мети застосували один із методів оцінювання антиоксидантної активності при ініціюванні вільнорадикальних процесів *in vitro*, а саме за інгібуванням накопичення активних форм кисню.

Результати. Дослідили двадцять три S-похідних 5-(тіофен-3-ілметил)-4-R-1,2,4-тріазол-3-тіолів. На першому місці за силою ефекту – сполука 13 натрію 2-((4-феніл-5-тіофен-3-ілметил)-1,2,4-тріазол-3-іл)тіо)етаноат що перевищує активність обох препаратів порівняння. Наступна за ступенем активності – сполука 22 1-феніл-2-((4-феніл-5-(тіофен-3-ілметил)-4*H*-1,2,4-тріазол-3-іл)тіо) етан-1-ол, що має наближений ефект до емоксипіну; дещо поступається йому сполука 7 1-(3-фторфеніл)-2-((5-тіофен-3-ілметил)-4*H*-1,2,4-тріазол-3-іл)тіо)етан-1-он.

Висновки. Окремі з нових S-похідних 1,2,4-тріазолу характеризуються значним антиоксидантним ефектом, що є наближеним чи перевершує ефективність референс-препаратів.

Ключові слова: 1,2,4-тріазол, антиоксидантна активність, супероксиддисмутаза.

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Modern science has demonstrated that the activation of lipid peroxidation processes significantly influences the development of various pathological conditions in the body, such as stress, atherosclerosis, myocardial infarction, hyperglycemia, and the formation of malignant tumors [1]. Lipid peroxidation leads to the formation and accumulation of highly toxic substances, which in turn potentiate the destabilization of cellular membranes and subcellular structures. Consequently, antioxidant drugs are increasingly included as a constant component in the complex pathogenetic therapy of various diseases.

For this reason, research of substances with antioxidant properties has gained great importance in the development of modern medicine and pharmacy.

Heterocyclic compounds attract more attention compared to monocyclic ones due to their wide range of pharmacological properties [2,3,4]. One of the promising heterocycles is the five-membered ring, namely 1,2,4-triazole. Among the derivatives of 1,2,4-triazole, several have been identified with high antioxidant properties. Therefore, we synthesized and studied a series of new thiophene derivatives of 1,2,4-triazole. Previously, we conducted preliminary pharmacological screening of the antioxidant activity of various classes of the derivatives that we have obtained. Namely, molecular docking, molecular dynamics simulations, and MM-PBSA calculations. Based on the screening results, we selected compounds for further in vitro studies [5].

Aim

To assess the antioxidant properties of newly synthesized S-derivatives of 1,2,4-triazole through the inhibition of reactive oxygen species accumulation in the superoxide dismutase system.

Materials and methods

All studies were conducted at Zaporizhzhia State Medical and Pharmaceutical University.

According to standard methodology, the non-enzymatic oxidation reaction occurs in an alkaline medium [6,7,8]. In a spectrophotometric cuvette, we added 2 ml of 0.15 M sodium carbonate buffer with the addition of EDTA-Na_2 solution, followed by the solutions of the compounds under investigation. The reaction was initiated by introducing an aqueous solution of adrenaline, prepared in doubly distilled water using only pure crystalline adrenaline. The prepared adrenaline solution was adjusted to pH 2.25 with hydrochloric acid. The reaction was conducted with an exposure time of 3 minutes, at a temperature of 35–36 °C, and a wavelength of 480 nm. Calculations were performed using the following formula:

$AOA = (D_x - D_o) / D_x \times 100$ (%),

where, D_x – is the optical density reflecting the rate of uninhibited auto-oxidation of adrenaline;

D_0 – is the optical density reflecting the rate of auto-oxidation of adrenaline in the presence of the studied compounds.

As reference drugs, we selected the classical potent antioxidants thiotriazoline and emoxipine.

Results

To achieve the stated objectives, we conducted preliminary pharmacological screening of antioxidant activity in vitro for twenty-three newly synthesized compounds. Among the studied compounds, there are S-derivatives belonging to various classes: alkyls, ketones, ethanoic acids and their salts, isopropyl esters of ethanoic acids, alcohols, and acetamides (*Table 1, Fig. 1*).

Among the studied compounds, different classes of S-derivatives exhibited varying effects on the level of superoxide radical inhibition in vitro: alkyl derivative (compound **1**), ketone derivatives (compounds **5** and **7**), salts (compounds **13** and **15**), acetamide (compound **20**), and alcohol (compound **22**) (*Tables 2, 3*). Notably, compound **13**, sodium 2-((4-phenyl-5-thiophene-3-ylmethyl)-1,2,4-triazole-3-yl) thio)acetate, demonstrated the highest antioxidant activity with a reduction of 72.0 %, surpassing the activity of both reference drugs.

Additionally, compound **22**, 1-phenyl-2-((4-phenyl-5-(thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)ethanol, exhibited a reduction of 60 %, showing activity similar to that of emoxipine.

The least pronounced effect, which did not exceed that of the reference drugs, was observed for the following compounds. Compound **7**, 1-(3-fluorophenyl)-2-((5-thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)ethanone, showed a reduction of 56.0 %. Similarly, the level of superoxide radical inhibition in vitro was decreased by 3-(pentylthio)-4-phenyl-5-(thiophene-3-ylmethyl)-1,2,4-triazole (compound **1**) by 40.0 % and N-methyl-2-((4-phenyl-5-(thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)acetamide (compound **20**) by 40 %. The smallest effect was recorded for compounds **5**, 1-(4-methoxyphenyl)-2-((4-phenyl-5-(thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio)ethanone, and **15**, calcium 2-((5-thiophene-3-ylmethyl)-4*H*-1,2,4-triazole-3-yl)thio) acetate, with reductions of 32.0 %.

The antioxidant activity of the reference drugs thiotriazoline and emoxipine was significant, with reductions in superoxide radical inhibition of 71.66 % and 62.5 %, respectively. Thus, among the compounds studied, only compound **13** exceeded the effects of thiotriazoline and emoxipine.

The results of the studies of the aforementioned compounds were statistically significant compared to the control group ($p < 0.05$).

Based on the research findings, certain patterns regarding the "structure-activity" relationship can be established. It is noteworthy, that the introduction of a phenyl radical at the fourth position of the triazole fragment increased the antioxidant activity. Additionally, the reduction of the ketone group to an alcohol significantly enhanced the antioxidant properties.

Among the other ketone derivatives, compound **7** exhibited the greatest impact on the process of inhibiting the oxidation of adrenochrome. This is likely associated with the positional change of the fluorine atom in the aromatic substituent.

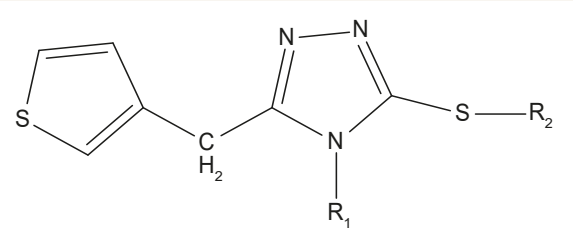

Fig. 1. General formula of S-derivatives of 5-(thiophene-3-ylmethyl)-4-R-1,2,4-triazole-3-thiols.

Table 1. Structure of some S-derivatives of 5-(thiophene-3-ylmethyl)-4-R-1,2,4-triazole-3-thiols

Compound	R_1	R_2
Compound 1	C_6H_5	C_5H_{11}
Compound 2	H	CH_3
Compound 3	H	C_5H_{11}
Compound 4	H	$C_{10}H_{21}$
Compound 5	C_6H_5	CH_2(CHO)-4-OMeC_6H_4
Compound 6	C_6H_5	CH_2(CHO)-3-FC_6H_4
Compound 7	H	CH_2(CHO)-3-FC_6H_4
Compound 8	H	CH2(CHO)-2-FC6H4
Compound 9	C_6H_5	C_4H_4S
Compound 10	C_6H_5	-CH_2-COOH
Compound 11	H	-CH_2-COOH
Compound 12	C_6H_5	-CH_2-COO$^-$ K^+
Compound 13	C_6H_5	-CH_2-COO$^-$ Na^+
Compound 14	C_6H_5	-CH_2-COO$^-$ Ca^{2+}
Compound 15	H	-CH_2-COO$^-$ Ca^{2+}
Compound 16	H	-CH_2-COO$^-$ Na^+
Compound 17	H	-CH_2-COO-CH$(CH_3)_2$
Compound 18	C_6H_5	-CH_2-CO-NH_2
Compound 19	H	-CH_2-CO-NH_2
Compound 20	C_6H_5	$NHCH_3$
Compound 21	C_6H_5	NHC_2H_5
Compound 22	C_6H_5	-CH2-CH(OH)-C_6H_5
Compound 23	H	-CH2-CH(OH)-C_6H_5

Discussion

The targeted search for new synthetic compounds with antioxidant properties necessitates their investigation in various experimental models of lipid peroxidation initiation [9,10]. This is primarily due to the activation of free radical oxidation of lipids, which is a key mechanism in the pathogenesis of most diseases [11].

We studied the antioxidant activity of novel thiophene-3-yl-methyl-substituted 1,2,4-triazoles through the inhibition of

Table 2. Effect of 1,2,4-triazole derivatives (10^{-6} M) on superoxide radical inhibition *in vitro* (n = 9)

Compound	The optical density of the superoxide-radical, Δ	Antioxidant activity, %
Control series	0.250 ± 0.001	–
Compound 1	0.150 ± 0.001*	40.0
Compound 2	0.250 ± 0.001	0
Compound 3	0.250 ± 0.001	0
Compound 4	0.250 ± 0.001	0
Compound 5	0.330 ± 0.001	+32.0
Compound 6	0.250 ± 0.001	0
Compound 7	0.110 ± 0.001*	56.0
Compound 8	0.250 ± 0.001	0
Compound 9	0.250 ± 0.001	0
Compound 10	0.270 ± 0.001	+8.0
Compound 11	0.250 ± 0.001	0
Compound 12	0.270 ± 0.001	+8.0
Thiotriazoline	0.068 ± 0.001*	71.66
Emoxipine	0.090 ± 0.001*	62.5

*: significance between the control series and the experimental group, $p < 0.05$.

Table 3. Effect of 1,2,4-triazole derivatives (10^{-6} M) on superoxide radical inhibition *in vitro* (n = 9)

Compound	The optical density of the superoxide-radical, Δ	Antioxidant activity, %
Control series	0.250 ± 0.001	–
Compound 13	0.070 ± 0.001*	72.0
Compound 14	0.250 ± 0.001	0
Compound 15	0.170 ± 0.001*	32.0
Compound 16	0.250 ± 0.001	0
Compound 17	0.280 ± 0.001	+12.0
Compound 18	0.250 ± 0.001	0
Compound 19	0.250 ± 0.001	0
Compound 20	0.350 ± 0.001*	+40.0
Compound 21	0.280 ± 0.001	+12.0
Compound 22	0.400 ± 0.001*	+60.0
Compound 23	0.250 ± 0.001	0
Control series	0.240 ± 0.001	–
Thiotriazoline	0.068 ± 0.001*	71.66
Emoxipine	0.090 ± 0.001*	62.5

*: significance between the control series and the experimental group, $p < 0.05$.

reactive oxygen species accumulation. This method is widely used in scientific research for screening antioxidant activity in vitro. It is based on the ability of physiologically active compounds to inhibit reactive oxygen species. In particular, we investigated how the tested compounds affect the rate of adrenaline auto-oxidation to adrenochrome (a colored product), which is accompanied by the accumulation of the free anion radical $O^{2\bullet}$.

Our findings highlight the importance of structural modifications in enhancing antioxidant activity, providing valuable insights for future research and development of effective antioxidant agents.

Conclusions

1. For the first time, the antioxidant activity of new derivatives of 5-(thiophene-3-ylmethyl)-4-R1-1,2,4-triazole-3-thiol has been studied. Several compounds were identified, that exhibit activity comparable to or exceeding that of the reference drugs emoxipine and thiotriazoline.

2. The most significant antioxidant activity was demonstrated by compound **13** (sodium 2-((4-phenyl-5-thiophene-3-ylmethyl)-1,2,4-triazole-3-yl)thio)acetate).

3. In some cases, patterns were established regarding the influence of different substituents on the antioxidant activity of the obtained compounds.

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