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BIOLOGICAL ACTIVITY AND TOXICOLOGICAL PROFILE OF DIACETATE DERIVATIVES OF 3,5-*BIS*(5-MERCAPTO-4-R-4*H*-1,2,4-TRIAZOL-3-YL)PHENOL

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Objective. The main objective of this study is to identify novel antioxidant compounds with high efficacy and low toxicity, synthesized on the basis of nitriles and diacetate and dibenzoic acid salts derived from 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol. The study also encompasses the confirmation of their purity, structure elucidation, and determination of their physicochemical characteristics.

Research materials. This article investigates the synthesis and establishes the structure of a series of compounds, specifically salts and nitriles of diacetate and dibenzoic acids of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol, which were selected as target materials for the search for highly efficient antioxidants.

Methods, research results, and their discussion. The research results demonstrated that several of the synthesized compounds possess significant antioxidant potential, which may be important for their application in various fields such as medicine, pharmaceuticals, and the chemical industry. Particular attention should be paid to 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))diacetonitrile (**compound 1c**) and 3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl)) bis(sulfanediyl))dipropanenitrile (**compound 2a**), which exhibited the highest level of antioxidant activity among all tested samples. These findings indicate the feasibility of using the investigated compounds as potential antioxidants in the manufacturing of materials and products, holding great significance for further scientific research.

Keywords: 1,2,4-triazole, synthesis, biological activity, antioxidant activity, DPPH.

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БІОЛОГІЧНА АКТИВНІСТЬ ТА ТОКСИКОЛОГІЧНИЙ ПРОФІЛЬ ДІАЦЕТАТНИХ ПОХІДНИХ 3,5-*БІС*(5-МЕРКАПТО-4-R-4*H*-1,2,4-ТРІАЗОЛ-3-ІЛ)ФЕНОЛУ

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Наукове дослідження відкриває перспективи для створення біологічно активних речовин на основі 3,5-*біс*(5-меркапто-4-R-4*H*-1,2,4-тріазол-3-іл)фенолу з антиоксидантною дією та низькою токсичністю. Зазначені АФІ мають потенціал для подальшого використання як самостійних, так і комбінованих препаратів для лікування патологічних станів, пов'язаних з оксидативним стресом.

Як перспективні антиоксидантні агенти були вибрані солі та нітрили діацетатних та дибензойних кислот, похідних 3,5-*біс*(5-меркапто-4-R-4*H*-1,2,4-тріазол-3-іл)фенолу. Застосовані методи включають органічний синтез, фізико-хімічне визначення будови (елементний аналіз, УФ-спектрофотометрія, ^{1}Н ЯМР-спектрометрія, хромато-мас-спектрометрія), а також біологічний метод оцінки АОА за допомогою DPPH та гострої токсичності сполуки-лідера.

Ключові слова: 1,2,4-тріазол, синтез, біологічна активність, антиоксидантна активність, DPPH.

Introduction

The modern landscape of the pharmaceutical market offers a wide range of active pharmaceutical ingredients (APIs) aimed at cellular and tissue protection, which is recognized as an essential component of healthcare. A striking example of this approach is the successful introduction of the drug Thiotriazoline and its derivatives, such as Thiocetam, Thiodarone, and Indotril, to the Ukrainian market. Pharmaceutical scientists at Zaporizhzhia State Medical and Pharmaceutical University, along with researchers from international academic institutions, are conducting studies to develop novel, highly effective, and low-toxicity 1,2,4-triazole derivatives [1–10].

In particular, our work aims to identify potent antioxidants [5] that could potentially be utilized as antihypoxants based on newly developed salts and nitriles

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synthesized from diacetate and dibenzoic acids derived from 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol [5].

The aim of the work. The study is aimed at searching for and identifying novel antioxidant compounds characterized by high efficacy and minimal toxicity [5; 6]. These compounds are structurally based on nitriles and salts synthesized from diacetate and dibenzoic acids derived from 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol. Antioxidants with such characteristics hold potential for applications in medicine [1–3; 5; 6], pharmaceuticals, and other fields requiring effective agents to counteract oxidative stress and associated diseases.

In addition to searching for novel compounds, the study focuses on confirming their purity and determining their molecular structure. An important aspect involves establishing the physicochemical characteristics of the synthesized compounds, which will enable a deeper understanding of their properties and application capabilities. A thorough analysis of the structural features and properties of the new compounds is essential for evaluating their potential as effective and safe antioxidants. This approach not only expands knowledge regarding the chemical properties of new 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol derivatives, but also paves the way for utilizing these compounds in future scientific and practical developments [5].

Materials and Methods

The article investigates the synthesis and structure of a series of chemical compounds – salts and nitriles of diacetate and dibenzoic acids derived from 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol. These compounds were selected due to their potential as effective antioxidants with prospective applications in veterinary and human medicine. Their synthesis opens up prospects for developing materials capable of protecting cells from oxidative stress, which is crucial for the design of novel therapeutic agents.

To elucidate the structure, organic synthesis and physicochemical analytical methods were employed, including elemental analysis, UV spectrophotometry, ^{1}H NMR, mass spectrometry, and gas chromatography-mass spectrometry (GC-MS). These methods confirmed the purity and targeted properties of the synthesized compounds.

The antioxidant activity was evaluated using a biological assay based on the free radical scavenging capacity (DPPH assay). Additionally, a bibliosemantic analysis of literature was conducted using Google Scholar, PubMed, Scopus, and Web of Science, ensuring the relevance and scientific validity of the findings.

Research results and their discussion

Determination of the antioxidant activity of 3,5-bis(5-mercapto-4-R-4H-1,2,4-triazol-3-yl)phenol derivatives

The antioxidant activity of the synthesized compounds was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay [5].

Our experimental data (*Table 1*) demonstrate that two compounds, namely 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetonitrile (**compound 1c**) and 3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))dipropanenitrile (**compound 2a**), possess a higher level of antioxidant activity than the reference drug, the natural antioxidant ascorbic acid. This indicates that the investigated compounds may be promising candidates for application as potent antioxidants. The high activity of these compounds may be attributed

Table 1

Antioxidant activity parameters of salts and nitriles derived from diacetate derivatives of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol

No.	Compound	Absorbance, *A*	Antioxidant activity, %
1	**DPPH control solution**	1.4054	–
2	**Ascorbic acid (reference)**	0.6847	**51.28**
3	**1a**	0.8280	41.08
4	**1b**	0.7548	17.16
5	**1c**	0.6391	**54.53**
6	**2a**	0.6763	**51.88**
7	**2b**	0.8318	40.81
8	**2c**	0.7896	43.82
9	**3a**	0.8847	37.05
10	**3b**	0.7302	19.86
11	**3c**	0.7737	44.95
12	**4a**	0.8284	41.06
13	**4b**	0.9093	35.30
14	**4c**	0.9008	35.90
15	**5a**	0.7955	12.70
16	**5b**	0.7685	15.66
17	**5c**	0.6354	30.27
18	**6a**	0.716	21.42
19	**6b**	0.7583	16.78
20	**6c**	0.6871	24.59

to the presence of electron-donating groups capable of donating electrons, which promotes the reduction of free radical activity by stabilizing them through hydrogen atom transfer or π-electron delocalization. Furthermore, the presence of a sulfur atom bonded to the 1,2,4-triazole ring may additionally enhance the antioxidant capacity of the compounds.

Considering the antioxidant activity exhibited by 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol derivatives presented in Table 1, these compounds possess significant potential for prospective use in combating oxidative stress-related diseases, such as neurodegenerative disorders, Alzheimer's disease, Parkinson's disease, and cardiovascular diseases. Further studies could evaluate their efficacy in protecting cells from oxidative damage, investigate their underlying mechanisms of action, and validate their potential as therapeutic agents. In particular, additional research should focus on their capacity to scavenge free radicals, attenuate inflammatory processes, and improve cellular state under stress conditions.

Given the antioxidant activity demonstrated by the 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol derivatives (*Table 1*), these compounds may hold promise for use in the treatment of diseases associated with oxidative stress. Future studies could focus on evaluating their ability to protect cells from oxidative damage, as well as investigating their potential as therapeutic agents in pathologies such as neurodegenerative and cardiovascular diseases [5].

The prospects of the chosen research direction are further supported by the analyzed literature [11–16].

Acute toxicity of 3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))dipropanenitrile

The safety profile of the most active antioxidant, 3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))dipropanenitrile (**compound 2a**), was evaluated in accordance with the international OECD Test Guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method). This approach allows for the verification of the toxicological category of a substance while significantly reducing the number of experimental animals required. The experimental study was conducted in compliance with bioethical standards (Directive 2010/63/EU) and received a favorable opinion from the Bioethics Committee of Zaporizhzhia State Medical and Pharmaceutical University (Minutes No. 2023-2phc dated February 14, 2023).

In accordance with the ARRIVE 2.0 guidelines, 15 sexually mature female Wistar rats weighing 180–220 g were selected for the study. The selection of females was based on their potentially higher sensitivity to xenobiotics compared to males. Animals were randomly allocated into experimental groups. Housing conditions complied with standard vivarium practices: a controlled temperature of 22 ± 2 °C, a 12-hour light/dark cycle, and ad libitum access to water. The experimental procedure involved step-by-step intragastric administration of the compound in the form of a suspension. Key survival metrics and the general clinical condition of the animals are presented in Table 2.

Analysis of the obtained data indicates that administration at doses of 5, 50, and 300 mg/kg was not accompanied by visible signs of intoxication (changes in the condition of skin, eyes, mucous membranes, etc.). Only at a dose of 2000 mg/kg were moderate behavioral reactions observed, manifesting as decreased somatomotor activity and somnolence during the first 24 hours post-exposure. Critical manifestations such as convulsions, tremors, diarrhea, or lethargy were absent across all groups. Over the 14-day observation period, body weight dynamics in animals remained stable (deviations < 5 %, $p > 0.05$), confirming the absence of prolonged toxic effects.

Based on the OECD TG 423 decision-making flowchart, taking into account the mortality of one animal at a dose of 300 mg/kg and two animals at 2000 mg/kg, **compound 2a** was classified as follows:

According to the Globally Harmonized System (GHS) and Regulation (EC) No. 1272/2008 (CLP), the substance belongs to Acute Oral Toxicity **Category 4**.

The estimated median lethal dose range is $300 < LD_{50} \leq 2000$ mg/kg.

For toxicological labeling purposes, the LD_{50} cut-off value was determined to be 1000 mg/kg.

Synthesis of biologically active compounds based on 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol derivatives

Synthesis of 2,2'-(((5-hydroxy-1,3-phenylene) bis(4-R-4H-1,2,4-triazole-5,3-diyl))bis(sulfanediyl)) diaceto(propane)nitriles

To thoroughly study this class of compounds, acetic and propionic acid nitriles derived from 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol were synthesized. **Compounds 1a–c** and **2a–c** (Fig. 1, *Table* 3) were prepared by alkylation of the starting 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenols obtained in previous studies [5]. The reaction was carried out in an anhydrous alcoholic medium using bimolar amounts of α-chloroacetonitrile or α-chloropropiononitrile in the presence of sodium hydroxide. Heating the reaction mixture to 60–65 °C resulted in the formation of a precipitate consisting of

Table 2

Acute toxicity parameters and survival rate of rats following administration of compound 2a (*n* = 3 per stage)

Dose level (mg/kg)	Experimental stage	Number of animals	Mortality (number of deaths)	Clinical signs
5	Initial step	3	0	No clinical signs observed
50	Initial step	3	0	No clinical signs observed
300	Initial step	3	1	Normal activity preserved
300	Control	3	0	Normal activity preserved
2000	Initial step	3	2	Hypodynamia, somnolence

acetic and propionic acid nitriles (Fig. 2, **compounds 1a–c** and **2a–c**). Upon completion of the reaction and cooling of the mixture, the precipitated products were filtered off and washed with ether for purification.

During the optimization of the synthetic conditions, alkylation of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenols without alkali metal solutions was attempted. To obtain pure products, a bimolar amount of anhydrous sodium acetate was added. Notably, the resulting acetic and propionic acid nitriles (**compounds 1a–c** and **2a–c**) showed no melting point depression after recrystallization, confirming their purity and stability. However, the addition of sodium hydroxide to the reaction mixture significantly increased the yields of the target products by more than 20 %. Therefore, this protocol was selected as the primary method for further experiments, as depicted in Figure 1.

The obtained **compounds 1a, c** are gray crystalline substances, while **1b** is a brown crystalline solid. All compounds are soluble in alkaline solutions, organic solvents, and mineral acid solutions. For purification, the compounds were recrystallized from ethanol.

The obtained **compounds 2a, b** are white crystalline substances, while **2c** is a gray crystalline solid. All of them are soluble in alkaline solutions, organic solvents, and mineral acid solutions. For purification, the compounds were recrystallized from ethanol.

Synthesis of 2,2'-(((5-hydroxy-1,3-phenylene)bis(4-R-4H-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))diacetic acid salts

Many hetarylcarboxylic acids [1–3], despite their high bioactivity, exhibit low aqueous solubility, which limits their application. Since key physiological processes occur in an aqueous environment, enhancing the solubility of such compounds is essential.

In this study, water-insoluble 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetic acids and their salts (**compounds 3–6 a–c**, Fig. 2) with organic and inorganic bases were synthesized to improve solubility.

Due to the dual activity of their components, these compounds hold significant potential as therapeutic agents.

The synthesis of salts with Zinc (Zn^{2+}) and Iron (Fe^{2+}) cations is justified by their optimal coordination properties, biogenicity, low toxicity, and ability to effectively modulate the biological activity of compounds, in contrast to many other metals.

The selection of organic cations (ethylammonium and diethylammonium) is reasoned by their moderate basicity, high nucleophilicity, and capability to vary the steric and electronic properties of the molecule, thereby allowing targeted modulation of the solubility, lipophilicity, and biological activity of the synthesized compounds.

Salts of 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetic acids with organic bases (ethylamine, diethylamine) were synthesized in methanol followed by filtration.

Fe(II) and Zn salts were prepared via the intermediate formation of sodium salts followed by interaction with metal sulfates.

Compound 3a is a pink crystalline solid, while **3b, c** are brown crystalline solids; all are soluble in water, alkaline solutions, and mineral acids, and were purified by recrystallization (water–ethanol, 1:1).

Compounds 4a–c are brown crystals exhibiting similar solubility and purification parameters.

Compounds 5a–c are brown crystals, sparingly soluble in water (soluble upon heating), purified by reprecipitation from an alkaline NaOH/KOH solution upon the addition of Fe(II) sulfate.

Compounds 6a, b are white crystals, and **6c** is a gray crystalline solid; they are sparingly soluble in water (soluble upon heating) and were purified by reprecipitation from an alkaline NaOH/KOH solution upon the addition of $ZnSO_4$.

Experimental section

Synthetic procedures and characterization: ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer (USA) using dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent and tetramethylsilane (TMS) as an internal standard. The ^{1}H NMR spectra of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenols and their derivatives were processed using the ADVASP 143 software.

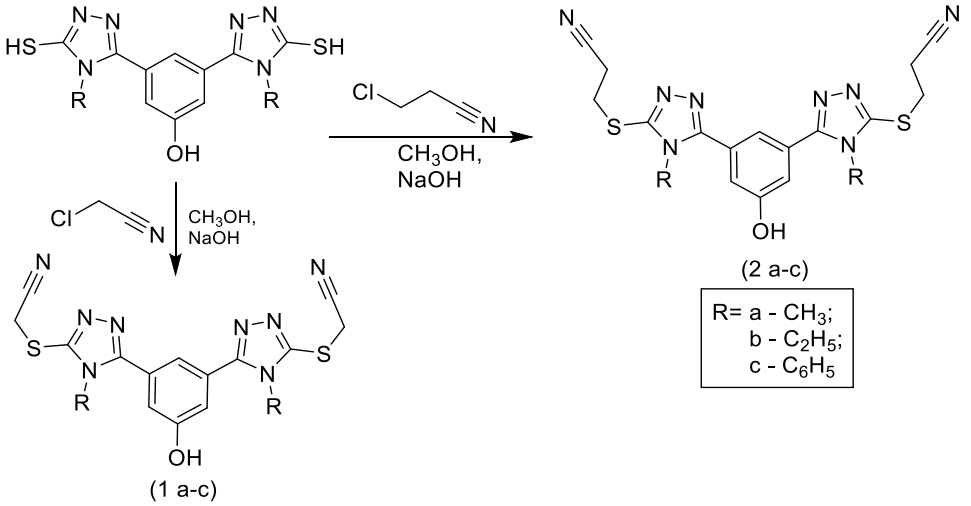

Fig. 1. Synthesis of 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl)) diaceto(propano)nitriles (compounds 1, 2a–c)

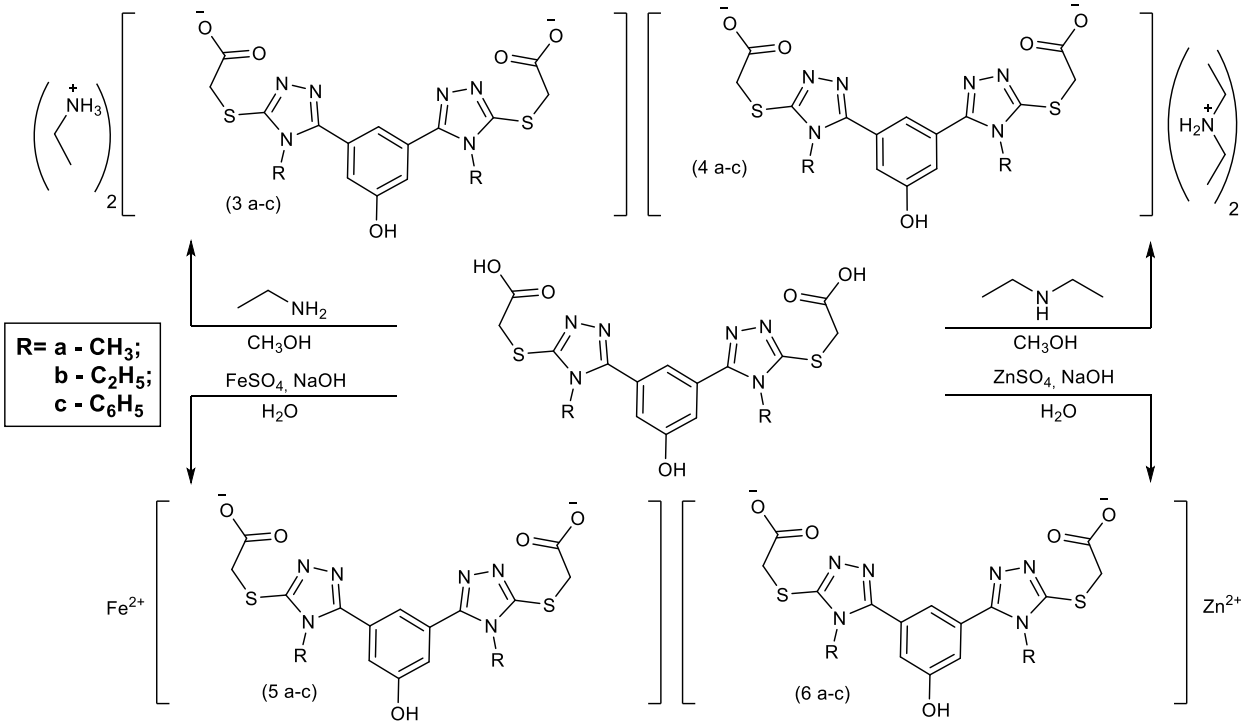

Fig. 2. Synthesis of 2,2'-(((5-hydroxy-1,3-phenylene)bis(4-R-4*H*-1,2,4-triazole-5,3-diyl))bis(sulfanediyl))diacetic acids (compounds 3–6 a–c)

Table 3

Physicochemical constants of the synthesized compounds 1, 2 a–c; 3–6 a–c

Compound	R	Anion	Yield (%)	MP (°C)	m/z
1a	CH_3	–	78	134–136	399
1b	C_2H_5	–	74	153–155	427
1c	C_6H_5	–	89	114–116	523
2a	CH_3	–	91	97–99	427
2b	C_2H_5	–	90	89–91	455
2c	C_6H_5	–	95	125–127	651
3a	CH_3	Fe^{2+}	79	291–293	437
3b	C_2H_5	Fe^{2+}	90	296–298	465
3c	C_6H_5	Fe^{2+}	91	390–392	561
4a	CH_3	Zn^{2+}	83	>400	437
4b	C_2H_5	Zn^{2+}	85	>400	465
4c	C_6H_5	Zn^{2+}	88	>400	561
5a	CH_3	$(C_2H_5NH_3^+)_2$	79	142–144	437
5b	C_2H_5	$(C_2H_5NH_3^+)_2$	77	121–123	465
5c	C_6H_5	$(C_2H_5NH_3^+)_2$	85	111–113	561
6a	CH_3	$((C_2H_5)_2NH_2^+)_2$	82	148–150	437
6b	C_2H_5	$((C_2H_5)_2NH_2^+)_2$	82	134–136	465
6c	C_2H_5	$((C_2H_5)_2NH_2^+)_2$	82	127–129	561

LC-MS analysis of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenols and their derivatives was performed using an Agilent 1260 Infinity HPLC System (USA) equipped with a degasser, a binary high-pressure pump, an autosampler, a column thermostat, and a diode-array detector, coupled with an Agilent 6120 single quadrupole mass spectrometer with electrospray ionization (ESI).

Molecular structure calculations were carried out using the AM1 semiempirical method (MOPAC 2003) following preliminary geometry optimization using the HyperChem® 10.0 software package.

Melting points of the synthesized compounds were determined in open capillary tubes using an MPA100 OptiMelt automated melting point system (USA) within a temperature range of 30–400°C with a resolution of 1 °C.

Elemental analysis of (3-thio-4-R-1,2,4-triazol-5-yl)(phenyl)methanols and their derivatives was performed on an ELEMENTAR vario EL cube analyzer (Germany) using sulfanilamide as a standard.

Compounds 1a–c: 2,2'-(((5-hydroxy-1,3-phenylene) *bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl)) diacetonitriles (Fig. 1, *Table 3*). A mixture of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol (0.01 mol; R = -CH_3, -C_2H_5, -C_6H_5) and sodium hydroxide (0.04 mol) in 50 mL of methanol was heated for 20 min (or until complete dissolution of the thiol). Subsequently, chloroacetonitrile (0.04 mol) was added to the reaction mixture, and the mixture was heated at 60 °C for 20 min (until a neutral pH was reached). The precipitate of formed sodium chloride was filtered off while the solution was hot. Upon complete cooling, the resulting precipitate of 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetonitriles (**compounds 1a–c**) was filtered off, washed with diethyl ether, and dried.

Compounds 2a–c: 2,2'-(((5-hydroxy-1,3-phenylene) *bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl)) dipropanenitriles (Fig. 1, *Table 3*). A mixture of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol (0.01 mol; R = -CH_3, -C_2H_5, -C_6H_5) and sodium hydroxide (0.04 mol) in 50 mL of methanol was heated for 20 min (or until complete dissolution of the thiol). Subsequently, chloropropanenitrile (0.04 mol) was added to the reaction mixture, and the mixture was heated at 60 °C for 20 min (until a neutral pH was reached). The precipitate of formed sodium chloride was filtered off while the solution was hot. Upon complete cooling, the resulting precipitate of 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl)) *bis*(sulfanediyl))dipropanenitriles (**compounds 2a–c**) was filtered off, washed with diethyl ether, and dried.

1a ^{1}H NMR (300 MHz, DMSO) δ 9.44 (s, 1H, OH), δ 7.45 (t, 1H, Ar), δ 7.22, (d, 2H, Ar), δ 4.40 (s, 4H, CH_2), δ 3.70 (s, 6H, S-CH_2). Deducted: C-45.57, H-3.11, N-24.66, S-14.11. Found: C-44.89, H-3.27, N-24.44, S-14.13.

1b ^{1}H NMR (300 MHz, DMSO) δ 9.42 (s, 1H, OH), δ 7.51 (d, 2H, Ar), δ 7.25 (t, 2H, Ar), δ 4.15 (s, 4H, CH_2), δ 4.15 (qu, 4H, CH_2), 1.33 (t, 6H, CH_3). Deducted: C-49.78, H-3.76, N-23.22, S-13.29. Found: C-49.40, H-3.73, N-23.29, S-13.36.

1c ^{1}H NMR (300 MHz, DMSO) δ 9.47 (s, 1H, OH), δ 7.60 (t, 2H, Ar), δ 7.45 (m, 9H, Ar), δ 7.20 (d, 2H, Ar), δ 4.19 (s, H, CH_2). Deducted: C-46.54, H-4.54, N-18.09, S-13.80. Found: C-47.01, H-4.59, N-17.90, S-13.77.

2a ^{1}H NMR (300 MHz, DMSO) δ 9.42 (s, 1H, OH), δ 7.60, (t, 1H, Ar), δ 7.21 (d, 2H, Ar), δ 3.70 (s, 6H, N-CH_3), δ 3.40 (t, 4H, S-CH_2), δ 2.92 (t, 4H, CH_2-CH_2). Deducted: C-50.69, H-4.25, N-26.27, S-15.03. Found: C-50.20, H-4.33, N-26.49, S-15.74.

2b ^{1}H NMR (300 MHz, DMSO) δ 9.39 (s, 1H, OH), δ 7.51 (t, 1H, Ar), δ 7.25 (d, 2H, Ar), δ 4.22 (qu, 4H, N-CH_2), δ 4.15 (t, 4H, S-CH_2), δ 3.05 (t, 4H, CH_2-CH_2), 1.50 (t, 6H, CH_2-CH_3). Deducted: C-52.85, H-4.88, N-24.65, S-14.11. Found: C-52.60, H-4.54, N-24.50, S-14.20.

2c ^{1}H NMR (300 MHz, DMSO) δ 9.33 (s, 1H, OH), δ 7.60 (t, 2H, Ar), δ 7.55 (t, 5H, Ar), δ 7.39 (t, 4H, Ar), δ 7.22 (d, 2H, Ar), δ 3.50 (t, 4H, S-CH_2), δ 2.94 (t, 4H, S-CH_2). Deducted: C-61.07, H-4.03, N-20.35, S-11.64. Found: C-61.34, H-4.25, N-20.81, S-11.60.

Compounds 3a–c, 4a–c: Ethylammonium and diethylammonium 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetates (Fig. 2, *Table 3*).

A mixture of 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetic acid (0.01 mol; R = -CH_3, -C_2H_5, -C_6H_5) and 0.02 mol of the corresponding organic base (ethylamine or diethylamine) in 50 mL of methanol was kept at room temperature for 24 h. The reaction products were filtered off, washed, and dried.

Compounds 5a–c, 6a–c: Zinc and Iron(II) 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))diacetates (Fig. 2, *Table 3*).

To a suspension of 2,2'-(((5-hydroxy-1,3-phenylene) *bis*(4-R-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl)) diacetic acid (0.01 mol; R = -CH_3, -C_2H_5, -C_6H_5) in 30 mL of water, sodium hydroxide (0.02 mol) was added. The mixture was dissolved upon heating, followed by the addition of 0.02 mol of ZnSO_4 or FeSO_4 solution. The resulting precipitates were filtered off, washed with ethanol, and dried to afford **compounds 5a–c and 6a–c**.

3a ^{1}H NMR (300 MHz, DMSO) δ 9.38 (s, 1H, OH), δ 7.49 (t, 1H, Ar), δ 7.18 (d, 2H, Ar), δ 4.43 (s, 4H, CH_2), δ 3.74 (s, 6H, S–CH_2). Deducted: C-45.57, H-3.11, N-24.66, S-14.11.

3b ^{1}H NMR (300 MHz, DMSO) δ 9.46 (s, 1H, OH), δ 7.56 (d, 2H, Ar), δ 7.19 (t, 2H, Ar), δ 4.10 (s, 4H, CH_2), δ 4.21 (qu, 4H, CH_2), 1.28 (t, 6H, CH_3). Deducted: C-49.78, H-3.76, N-23.22, S-13.29.

3c ^{1}H NMR (300 MHz, DMSO) δ 9.50 (s, 1H, OH), δ 7.66 (t, 2H, Ar), δ 7.41 (m, 9H, Ar), δ 7.15 (d, 2H, Ar), δ 4.23 (s, 1H, CH_2). Deducted: C-46.54, H-4.54, N-18.09, S-13.80.

4a ^{1}H NMR (300 MHz, DMSO) δ 9.41 (s, 1H, OH), δ 7.43 (t, 1H, Ar), δ 7.28 (d, 2H, Ar), δ 4.38 (s, 4H, CH_2), δ 3.68 (s, 6H, S–CH_2). Deducted: C-45.57, H-3.11, N-24.66, S-14.11.

4b ^{1}H NMR (300 MHz, DMSO) δ 9.35 (s, 1H, OH), δ 7.48 (d, 2H, Ar), δ 7.31 (t, 2H, Ar), δ 4.19 (s, 4H, CH_2), δ 4.11 (qu, 4H, CH_2), 1.38 (t, 6H, CH_3).

4c ^{1}H NMR (300 MHz, DMSO) δ 9.52 (s, 1H, OH), δ 7.55 (t, 2H, Ar), δ 7.49 (m, 9H, Ar), δ 7.26 (d, 2H, Ar), δ 4.16 (s, 1H, CH_2). Deducted: C-46.54, H-4.54, N-18.09, S-13.80.

5a ^{1}H NMR (300 MHz, DMSO) δ 9.40 (s, 1H, OH), δ 7.50 (t, 1H, Ar), δ 7.19 (d, 2H, Ar), δ 4.45 (s, 4H, CH_2), δ 3.65 (s, 6H, S–CH_2). Deducted: C-45.57, H-3.11, N-24.66, S-14.11

5b ^{1}H NMR (300 MHz, DMSO) δ 9.48 (s, 1H, OH), δ 7.46 (d, 2H, Ar), δ 7.30 (t, 2H, Ar), δ 4.13 (s, 4H, CH_2),

δ 4.20 (qu, 4H, CH_2), 1.36 (t, 6H, CH_3). Deducted: C-49.78, H-3.76, N-23.22, S-13.29

5c ^{1}H NMR (300 MHz, DMSO) δ 9.43 (s, 1H, OH), δ 7.54 (t, 2H, Ar), δ 7.49 (m, 9H, Ar), δ 7.18 (d, 2H, Ar), δ 4.17 (s, 1H, CH_2). Deducted: C-46.54, H-4.54, N-18.09, S-13.80

6a ^{1}H NMR (300 MHz, DMSO) δ 9.39 (s, 1H, OH), δ 7.44 (t, 1H, Ar), δ 7.26 (d, 2H, Ar), δ 4.37 (s, 4H, CH_2), δ 3.77 (s, 6H, S–CH_2). Deducted: C-45.57, H-3.11, N-24.66, S-14.11

6b ^{1}H NMR (300 MHz, DMSO) δ 9.45 (s, 1H, OH), δ 7.55 (d, 2H, Ar), δ 7.29 (t, 2H, Ar), δ 4.18 (s, 4H, CH_2), δ 4.09 (qu, 4H, CH_2), 1.29 (t, 6H, CH_3). Deducted: C-49.78, H-3.76, N-23.22, S-13.29

6c ^{1}H NMR (300 MHz, DMSO) δ 9.51 (s, 1H, OH), δ 7.57 (t, 2H, Ar), δ 7.48 (m, 9H, Ar), δ 7.23 (d, 2H, Ar), δ 4.14 (s, 1H, CH_2). Deducted: C-46.54, H-4.54, N-18.09, S-13.80

Biological evaluation

Antioxidant activity: The antioxidant activity was determined using the DPPH assay [5]. A stock solution of the test compound (0.001 M) was prepared in 25 mL of DMSO and subsequently diluted to obtain a 0.0001 M solution. An aliquot of 2.00 mL of this solution was mixed with 2.00 mL of a 0.1 mM methanolic solution of DPPH (Sigma-Aldrich, Germany). The reaction mixture was incubated for 30 min in the dark at room temperature, and the absorbance was measured at 516 nm.

The control sample contained 2.00 mL of DPPH solution and 2.00 mL of methanol. Ascorbic acid was used as a reference standard. The free radical scavenging activity was calculated as percentage inhibition using the following formula:

$$\%\ of\ antioxidant\ activity = \frac{(A_0 - A_1)}{A_0} \cdot 100,$$

where A_0 is the absorbance of the control sample, and A_1 is the absorbance of the test sample. The absorbance of the test solutions was measured in aqueous-organic media by recording the absorption maximum at 516 nm using a Lambda 365 spectrophotometer.

Acute toxicity: The study was conducted on healthy female Wistar rats (aged 8–12 weeks, weighing 180–210 g), which are more sensitive than males. The animals were fasted overnight prior to dosing (with free access to water); food was withheld for an additional 3–4 h post-administration.

Housing conditions: Temperature was maintained 22 ± 3 °C, relative humidity at 50–60 % (range 30–70 %), with a 12/12 h light/dark cycle. Animals received standard diet and water *ad libitum*. The acclimatization period was at least 5 days.

Solutions of the test compounds were prepared immediately prior to administration and administered intragastrically via a gavage tube. Each testing step involved 3 animals. The initial starting dose was 5 mg/kg, followed by sequential doses of 50, 300, and 2000 mg/kg. If mortality occurred in 0–1 animals, the dose was repeated to confirm the results.

Observations: Animals were observed individually during the first 30 min, periodically throughout the first 24 h (with special emphasis during the first 4 h), and daily thereafter for a total observation period of 14 days.

Conclusions

1. Seventeen new compounds, including salts and nitriles derived from diacetic acid derivatives of 3,5-*bis*(5-mercapto-4-R-4*H*-1,2,4-triazol-3-yl)phenol, were synthesized.
2. The structures of the synthesized molecules were confirmed by a combination of physicochemical analytical methods, including ^{1}H NMR spectroscopy, elemental analysis, and ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS).
3. The antioxidant activity parameters for all target compounds were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.
4. Among the synthesized molecules, compounds exhibiting a high level of antioxidant capacity were identified.
5. The most prominent antioxidant effect was demonstrated by 2,2'-(((5-hydroxy-1,3-phenylene)*bis*(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl)) diacetonitrile (**compound 1c**) and 3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl)) *bis*(sulfanediyl))dipropanenitrile (**compound 2a**).
6. According to the GHS and Regulation (EC) No. 1272/2008 (CLP), **compound 2a** (3,3'-(((5-hydroxy-1,3-phenylene)*bis*(4-methyl-4*H*-1,2,4-triazole-5,3-diyl))*bis*(sulfanediyl))dipropanenitrile) belongs to Category 4 of acute oral toxicity ($300 < LD_{50} \leq 2000$ mg/kg), with an established LD_{50} cut-off value of 1000 mg/kg.

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