

## Novel bis([triazolo[3,4-*b*]thiadiazoles and bis([triazolo[3,4-*b*][thiadiazines) with antioxidant activity

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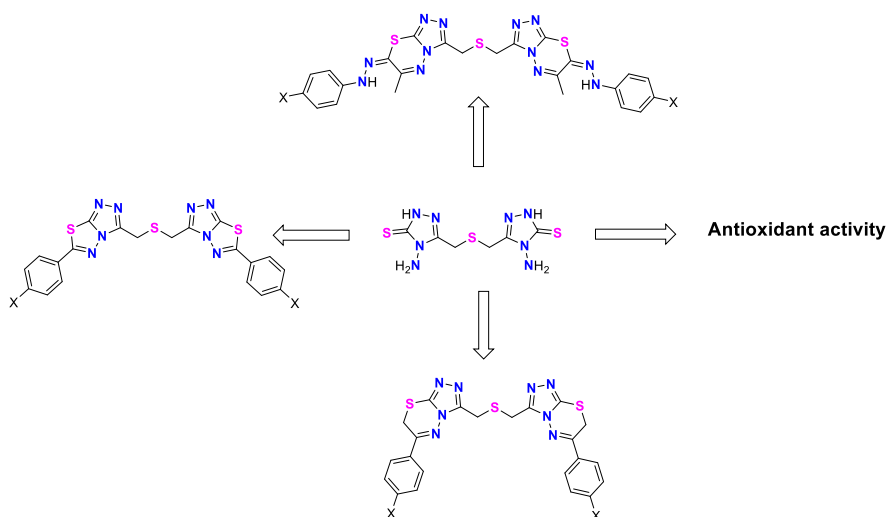
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### Abstract

A novel bis-triazole was synthesized in this context, and its potential usage as a flexible precursor for new bis-triazolothiadiazines and bis-triazolothiadiazoles with antioxidant properties was reported. The novel compounds' structures were determined using elemental analysis and spectrum data. According to the DPPH assay results, the newly synthesized compounds have noteworthy antioxidant characteristics.



**Keywords:** Bis(4-amino-4*H*-1,2,4-triazole-3-thiol), bis-triazolo[3,4-*b*][1,3,4]thiadiazoles, bis([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines), antioxidant activity

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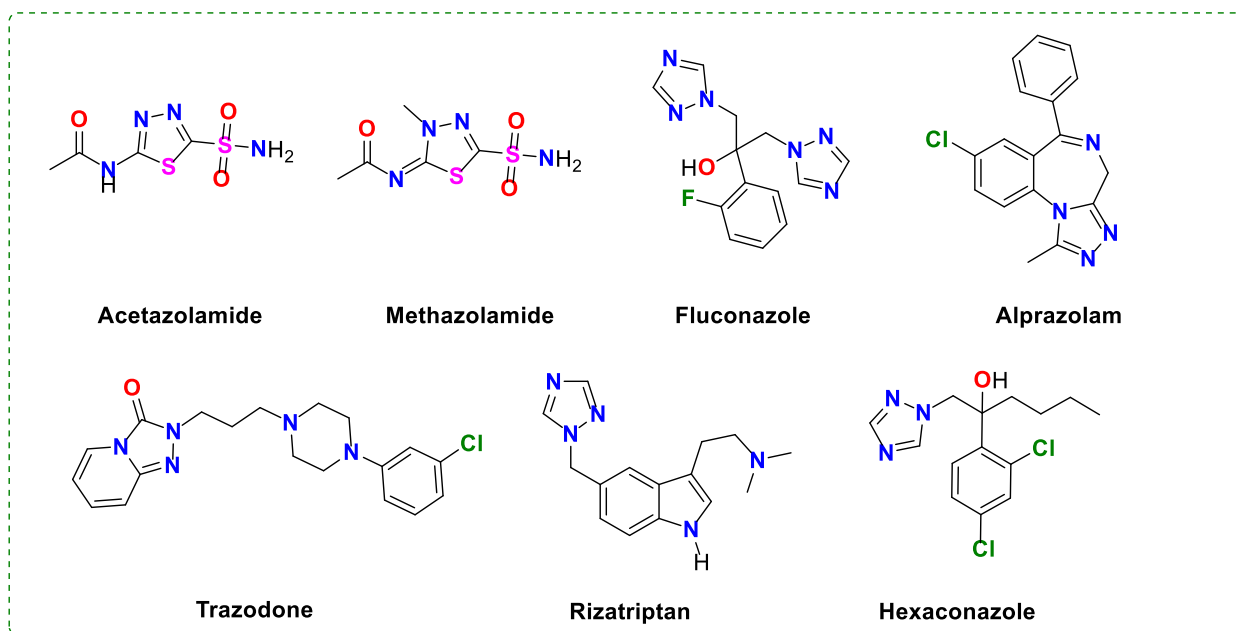
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## Introduction

Antioxidants are thought to be the first line of defense against free radical damage to living cells caused by biomolecular free radicals. As a result, great emphasis has been placed on the creation of synthetic antioxidants from a medicinal standpoint, particularly given today's increasing exposure to free radical sources owing to pollution, cigarette smoke, medications, disease, stress, and even exercise.<sup>1–3</sup> As a result, discovering novel synthetic molecules with antioxidant properties has become a significant problem for those interested in synthetic and medicinal chemistry. In this context, a large variety of heterocyclic compounds, particularly those containing sulfur and nitrogen, have recently been shown to exhibit antioxidant characteristics.<sup>4–6</sup> Among several synthesized compounds, derivatives of 1,2,4-triazoles<sup>7–9</sup>, 1,3,4-thiadiazoles<sup>10</sup>, and 1,3,4-thiadiazoles<sup>11,12</sup> have been shown to have noteworthy antioxidant properties. Moreover, various 1,2,4-triazoles, 1,3,4-thiadiazole, and 1,3,4-thiadiazene have been shown to show a variety of bioactivities, including neuroprotective, antimalarial, antileishmanial, antiviral, and anticonvulsant<sup>13–15</sup>. Many drugs containing 1,2,4-triazole and 1,3,4-thiadiazole moieties, such as acetazolamide (a diuretic and anticonvulsant), fluconazole (antifungal), methazolamide (carbonic anhydrase inhibitors), trazodone (an antidepressant), rizatriptan (an analgesic for headache treatment), hexaconazole (an antifungal agent), and alprazolam (sedative and tranquilizer) are included in figure 1.

In addition, triazolothiadiazole and triazolothiadiazine, a hybrid nucleus formed by the fusion of triazole with thiadiazole or thiadiazine, are essential nuclei due to their wide range of applications as promising pharmaceuticals<sup>16–18</sup>. They could be prepared from 4-amino-1,2,4-triazole-3-thiol utilizing the nucleophilic character of its amino and mercapto groups.<sup>19,20</sup>

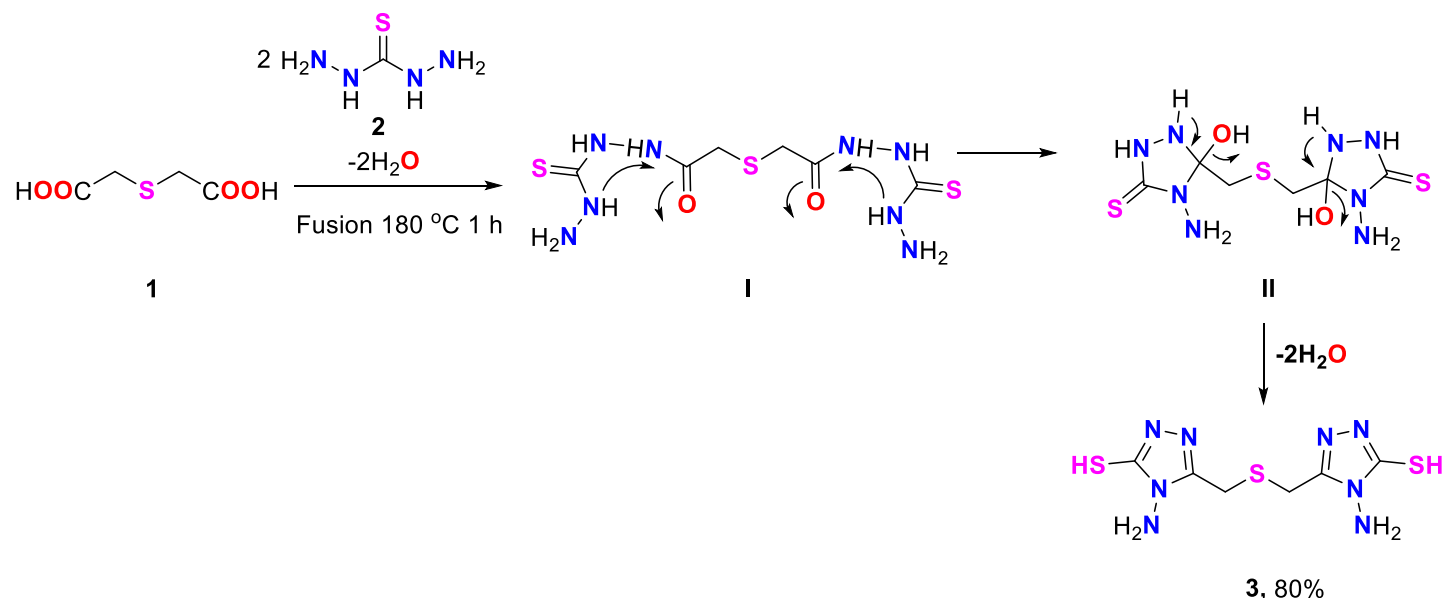


**Figure 1.** Some drugs containing 1,2,4-triazole and 1,3,4-thiadiazole moieties.

In light of these findings, and as part of our ongoing research on the synthesis of bis-heterocyclic derivatives<sup>21–31</sup> as prevalent scaffolds used in pharmaceutically important molecules<sup>32–46</sup>, we present herein the synthesis of novel bis-triazolothiadiazines and bis-triazolothiadiazoles linked by a pharmacophoric thioether bridge<sup>47</sup> from a novel bis-triazole precursor. The antioxidant capacity of the novel compounds was also tested.

## Results and Discussion

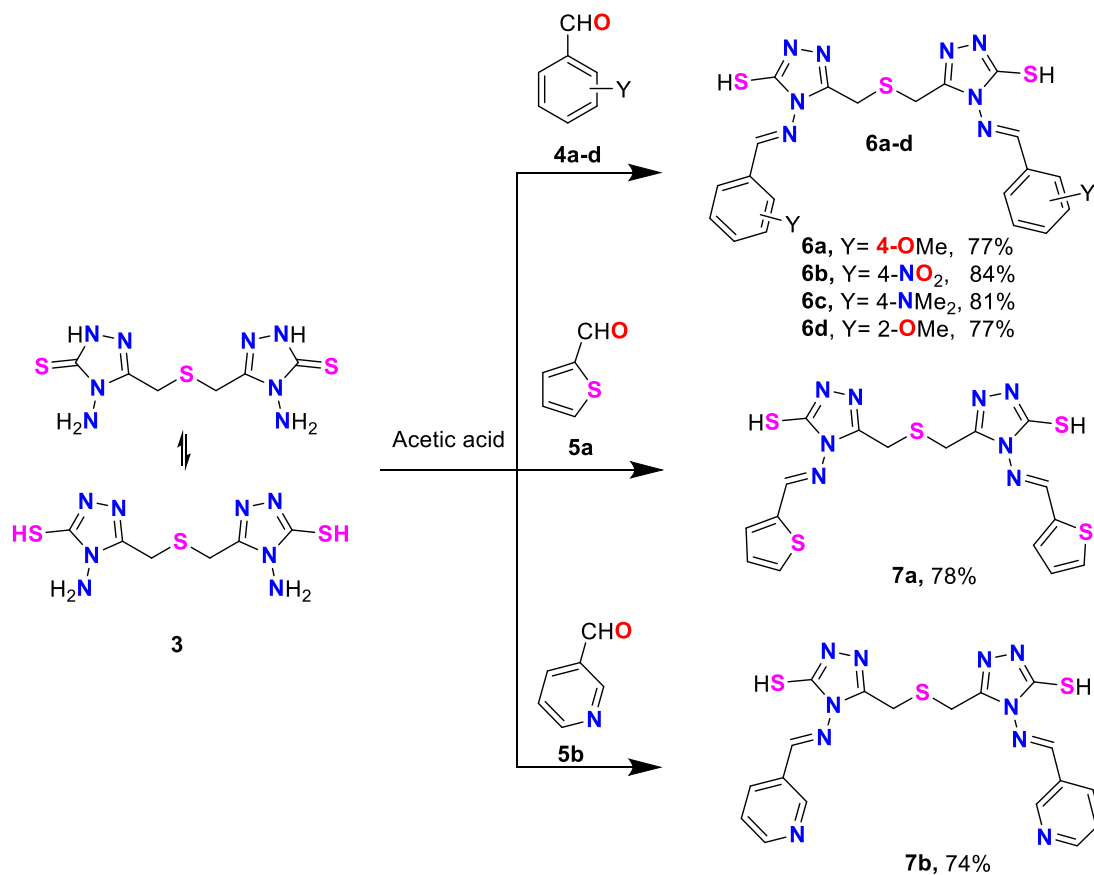
Bis(4-amino-4*H*-1,2,4-triazole-3-thiol) (**3**) was selected as a versatile precursor for the desired fused triazoles. It produced in an 80% yield by reacting one mole of 2,2'-thiodiacetic acid **1** with two moles of thiocarbonylhydrazide (**2**) under fusion conditions. The reaction begins with the elimination of two moles of water to form 2,2'-thiobis(*N*'-(hydrazinecarbonothioyl)acetohydrazide) **I**, which is then cyclized by nucleophilic attack of the NH-group on the carbonyl carbon to yield bis(4-amino-5-hydroxy-1,2,4-triazolidine-3-thione) **II**. Two moles of water were then eliminated, yielding the desired bis(4-amino-4*H*-1,2,4-triazole-3-thiol) (**3**) (Scheme 1).



**Scheme 1.** Synthesis of 5,5'-(thiobis(methylene))bis(4-amino-4*H*-1,2,4-triazole-3-thiol) (**3**).

Elemental analyses and spectral data were used to establish the structure of **3**. IR bands at 3293-3170  $\text{cm}^{-1}$  were identified as amine groups. Characteristic singlet signals for the  $\text{SCH}_2$  and  $\text{NH}_2$  protons were detected in its  $^1\text{H-NMR}$  spectrum in  $\text{DMSO-}d_6$  solution at 3.83, and 5.52 ppm, respectively. It also revealed a characteristic signal at  $\delta$  13.60 indicating the preference of the thiol over the thione form.<sup>48</sup>

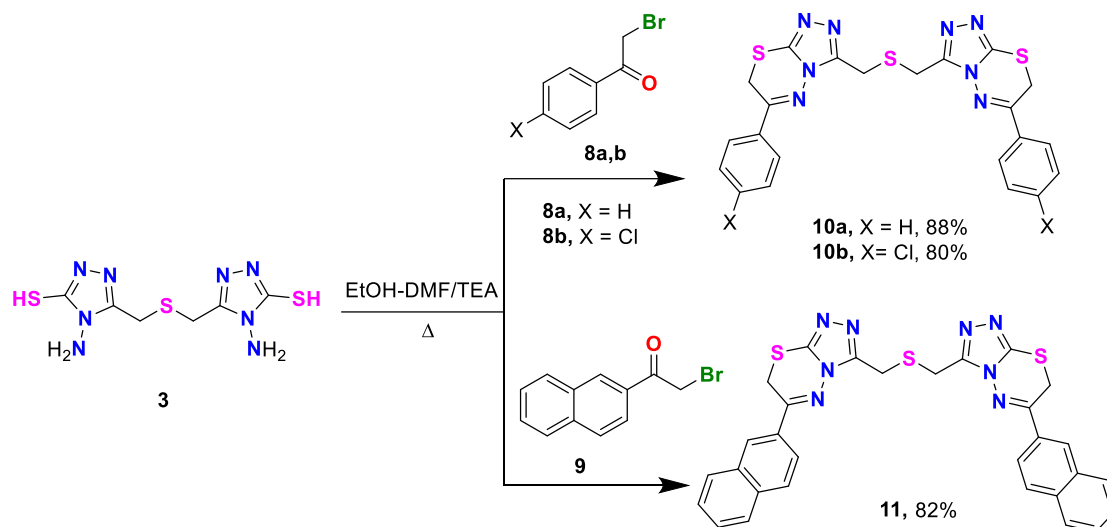
The synthetic utility of the bis-amine **3** as a building unit for a variety of heterocyclic systems was studied according to previous investigations.<sup>49,50</sup> The reactivity of bis-amine **3** to the production of new Schiff bases was first explored. Schiff base ligands are easily synthesized by the nucleophilic addition reaction of aldehydes or ketones with primary amines, which occurs mostly in the presence of an acid catalyst. Because of their many uses, they are unique substances in coordination chemistry, analytical chemistry, catalysis, and medicinal chemistry.<sup>51,52</sup> Thus, the reaction of **3** with *p*-methoxybenzaldehyde **4a** in glacial acetic acid under reflux yielded the corresponding bis(Schiff base) **6a** in 77% yield. Similarly, the reactions of **3** with 4-(dimethylamino)benzaldehyde **4b**, 4-nitrobenzaldehyde **4c**, *o*-methoxybenzaldehyde **4d**, thiophene-2-carbaldehyde **5a**, and nicotinaldehyde **5b** gave the corresponding bis(Schiff bases) **6b-d** and **7a,b**, respectively, in good yields (Scheme 2). As shown, heterocyclic aldehydes and aromatic aldehydes with substituents bearing either electron-donating or electron-withdrawing groups reacted well and had good product yields.



**Scheme 2.** Synthesis of 5,5'-(thiobis(methylene))bis(4-(arylideneamino)-4H-1,2,4-triazole-3-thiols) **6a-d** and **7a,b**.

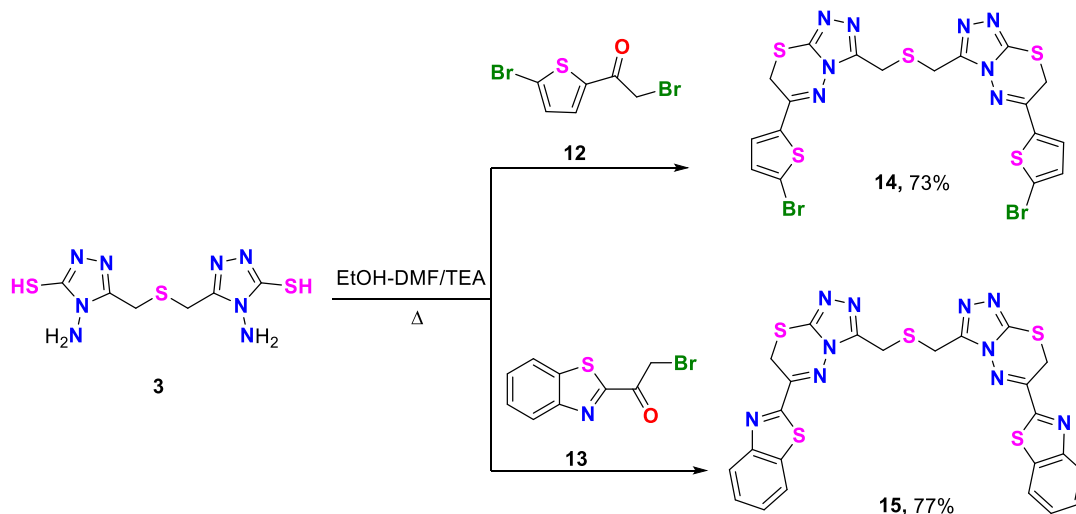
The reaction of NH<sub>2</sub> with the -CHO groups of the aldehydes, resulting in the formation of the corresponding Schiff bases, was confirmed by the disappearance of the NH<sub>2</sub> absorption bands in both the IR and <sup>1</sup>H-NMR spectra of **6a**. The <sup>1</sup>H-NMR spectrum of compound **6a** showed singlet signals at 3.79, 3.91, and 13.90, which belonged to the -OCH<sub>3</sub>, -SCH<sub>2</sub>, and -SH groups, respectively. In addition to the typical benzylidene signal at δ 9.73, the <sup>1</sup>H-NMR spectrum of **6a** also showed additional signals at 7.03–7.76 caused by aromatic ring protons derived from the aldehyde moiety.

The corresponding bis([(1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes **10a** and **10b** were produced in 88, and 80% yields, respectively, by treating **3** with two equivalents of 2-bromo-1-phenylethanone **8a** or 2-bromo-1-(4-chlorophenyl)ethanone **8b**, respectively. Similarly, when **3** was reacted under the same conditions with two equivalents of 2-bromo-1-(naphthalen-2-yl)ethanone **9**, 82% bis([(1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfane **11** was produced (Scheme 3).



**Scheme 3.** Synthesis of bis((7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfanes **10a,b** and **11**.

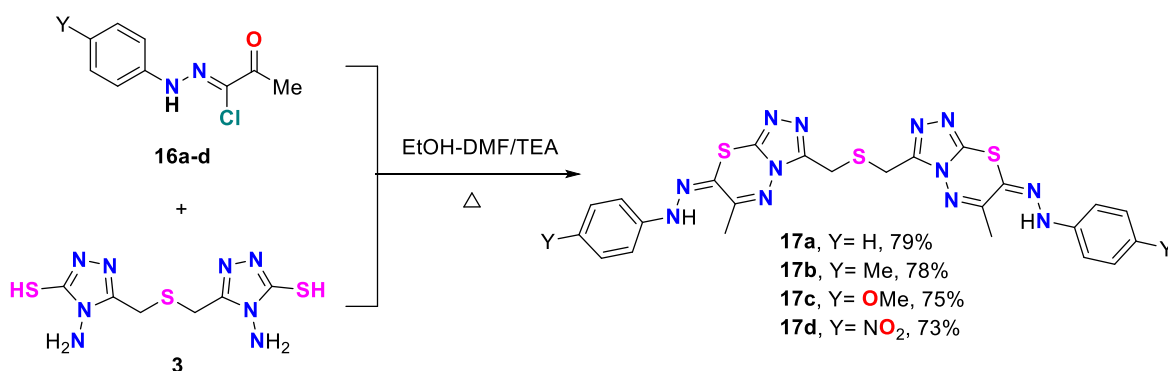
Using the same approach, we successfully synthesized novel bis((1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfanes **14** and **15** in 73 and 77% yields, where triazolothiadiazine is linked to heterocyclic moieties, by treating **3** with two equivalents of 2-bromo 1-(5-bromothiophen-2-yl)ethanone **12** and 1-(5-(benzo[d]thiazol-2-yl))-2-bromoethanone **13** at reflux in an EtOH/DMF mixture containing piperidine (Scheme 4).



**Scheme 4.** Synthesis of bis((1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfanes **14** and **15**.

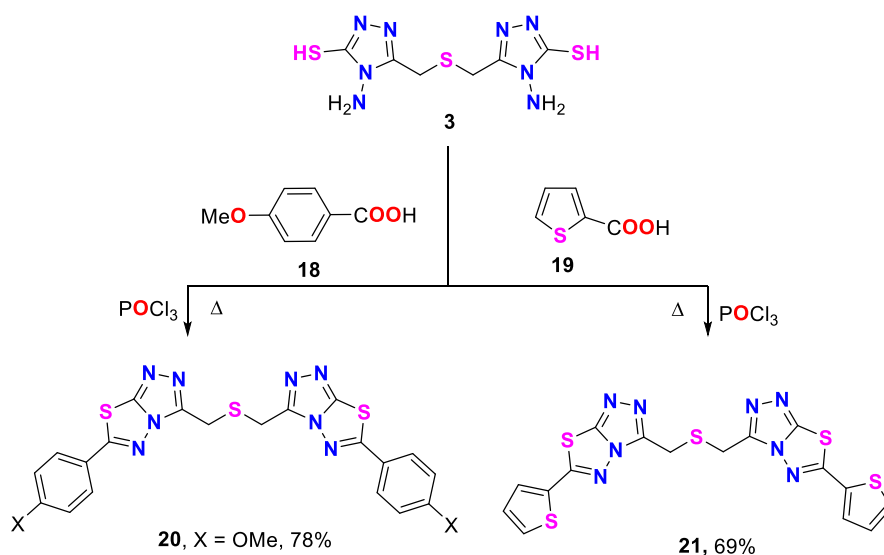
The spectral data and elemental studies utilized to characterize each isolated compound corroborated the suggested structures of **14** and **15**. Thus, the absence of the NH<sub>2</sub> stretching bands in the IR spectrum of triazolothiadiazine **14** and the lack of the main amine signal in their <sup>1</sup>H-NMR spectra supported the cyclocondensation of the suitable  $\alpha$ -bromoketone **12** with bis-aminotriazole **3**. The existence of thiadiazine ring SCH<sub>2</sub> protons that resonated at 4.10 ppm as singlet signals integrating four protons further confirmed the occurrence of a ring-closure process. Furthermore, the linker's SCH<sub>2</sub> protons resonated at 4.39 ppm as a singlet signal integrating four protons. All the other protons showed the expected chemical shifts and integral values (See Experimental section).

In refluxing EtOH containing triethylamine as a base, compound **3** was allowed to react with 2-oxo-N'-phenylpropanehydrazonoyl chloride **16a-d**. Reflux was carried out for 4-8 hours (monitored by thin-layer chromatography (TLC)) to yield bis((1,2,4)triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes **17a-d** in 73–79% yields (Scheme 5). The structures of these products were determined using elemental analysis and spectral data. The  $^1\text{H}$  NMR spectrum of compound **17d** as a representative example showed a characteristic singlet near 10.88, which was assigned to the NH of hydrazone, as well as singlet signals at 4.12 and 2.46, which were assigned to the  $-\text{SCH}_2$  and  $\text{CH}_3$  groups. The IR spectra revealed one band at  $3188\text{ cm}^{-1}$  assigned to the NH group and the absence of the absorption band assigned to the  $\text{NH}_2$  group.



**Scheme 5.** Synthesis of bis((7-(2-arylhydrazono)-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes **17a-d**.

Our study was extended to include the synthesis of bis((1,2,4)triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl)sulfanes **20** by reacting compound **3** with 4-methoxybenzoic acid **18** in a molar ratio (1:2) in the presence of phosphorous oxychloride, as shown in Scheme 6. Similar to the previous reaction, thiophene-2-carboxylic acid **19** and compound **3** were used to create bis([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl)sulfane **21** in 69% yield. (Scheme 6).

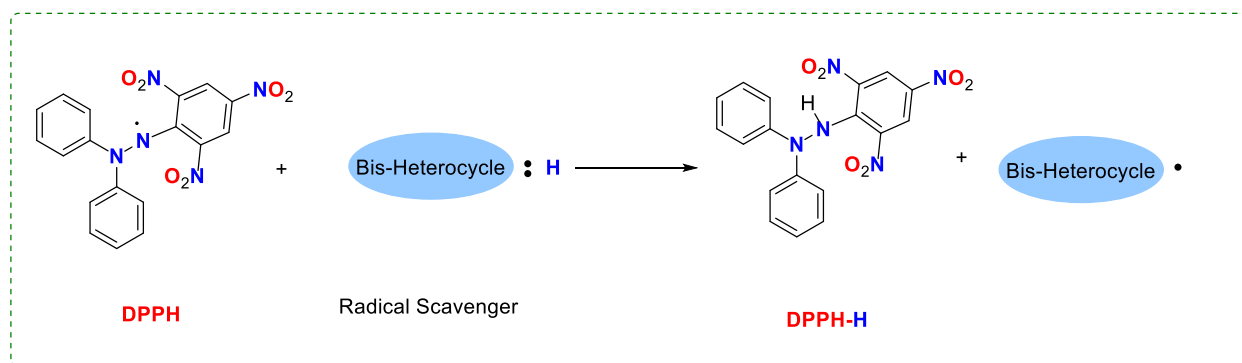


**Scheme 6.** Synthesis of bis([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl)sulfanes **20** and **21**.

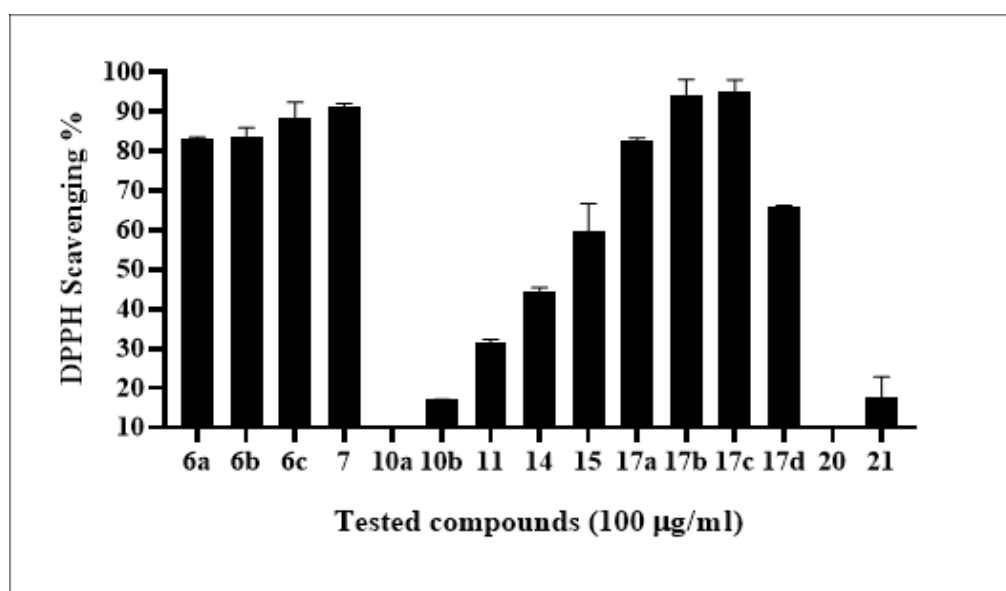
Both elemental analysis and spectral data were used to establish the structures of the products. Thus, for compound **20**, in addition to two doublets at 7.06 and 7.79 that are typical for aromatic protons, the  $^1\text{H}$  NMR spectrum of compound **20** also displayed a characteristic singlet signal integrating four protons at  $\delta$  4.38 and assigned to the  $\text{SCH}_2$  of the linker. Furthermore, the IR spectrum of **20** demonstrated a lack of absorption bands corresponding to the  $\text{NH}_2$  or  $\text{NH}$  groups.

### Antioxidant activity

In the DPPH experiment, an odd electron exhibits a significant absorption band at 519 nm, which loses absorbance after the odd electron is paired off by a hydrogen or electron-donating antioxidant (Figure 2). Based on the preliminary screening of tested compounds at a single concentration for determination of their ability for DPPH scavenging activity, ten compounds were found to show percentages higher than 50% of DPPH scavenging activity as shown in Fig. 3. These compounds were selected for  $\text{IC}_{50}$  (the concentration of test sample that causes 50% quenching of the UV absorption of DPPH) calculations. According to  $\text{IC}_{50}$  estimations, the most active compounds are **6b** and **7**, followed by **6a** and **6c**. The structure of the compound and its substituted groups greatly influence activity<sup>53</sup>.



**Figure 2.** Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant.

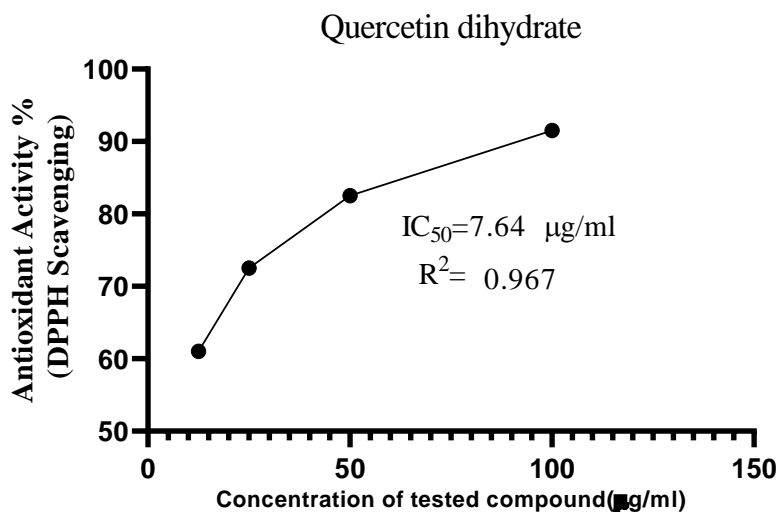


**Figure 3.** Primary screening of tested compounds for their antioxidant activity at a single concentration (100  $\mu\text{g}/\text{ml}$ ).

In the case of the DPPH assay, the means of the estimated % AA values obtained for the tested compounds were plotted against their respective concentrations, and the data were generated using a non-linear regression (concentration-response) curve fit, from which the  $IC_{50}$  values were derived (Table 1, cf supplementary file). Figures 4 and 5 show the concentration-response curve of DPPH scavenging by the positive control quercetin dihydrate.

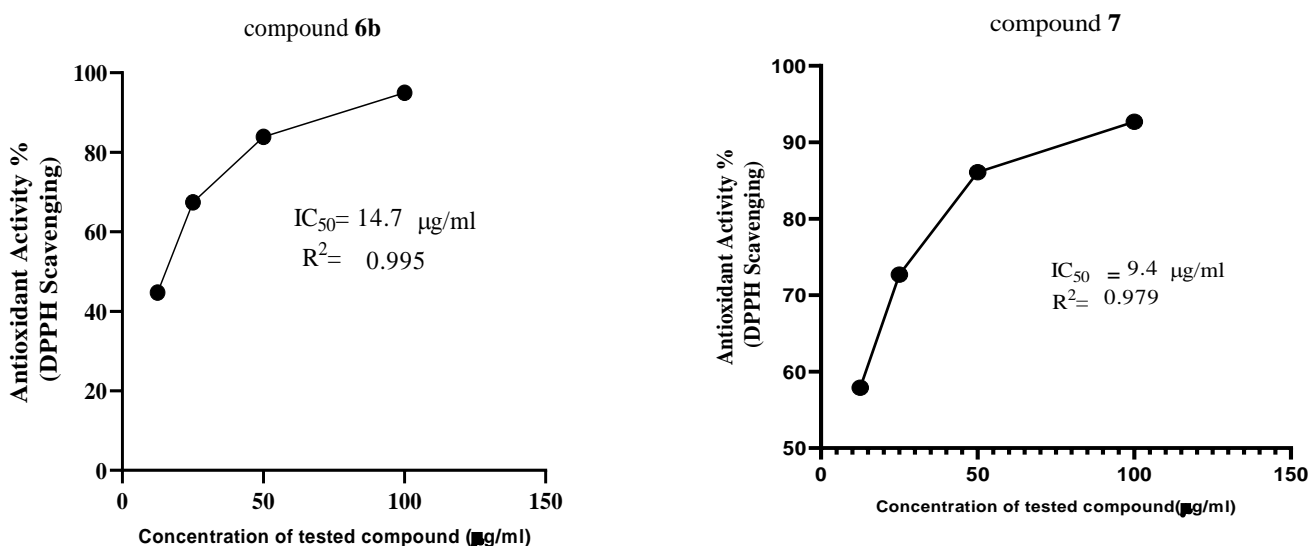
### Structure-activity relationship

According to the DPPH assay results, compounds (**6b** and **7**) have high antioxidant activity with low  $IC_{50}$  values. The donation of the first hydrogen from the SH group scavenges one DPPH radical in compounds **6a-c** with free SH group, converting the thiol to a Sulfur radical, which is stabilized by resonance with the imine group and provides electron supplementation, increasing radical scavenging ability. While the activities of derivatives with electron-donating substituents were comparable, the dimethylamino derivative was found to be much less effective than the methoxy derivative. Derivative **6b**, on the other hand, with a larger inductive action, improves hydrogen atom donating capacity. The activity of compound **3** was significantly reduced when it was converted to the matching fused derivatives. This can be explained by the loss of SH bonds during ring formation. Compound **17c**, on the other hand, with a free NH group and an electron-donating methoxy substituent at the para position of the aromatic ring, demonstrated modest activity. This implies that the loss of the hydrogen atom on the nitrogen atom linked to the aromatic ring, coupled with resonance stabilization, is significant in the free radical scavenging mechanism.



**Figure 4.** Concentration-response curve of DPPH scavenging by the positive control quercetin dihydrate.





**Figure 5.** Concentration-response curve of DPPH scavenging by most active compounds **6b** and **7**.

## Conclusions

Using a variety of reagents and reaction conditions, we synthesized a novel bis(4-amino-4H-1,2,4-triazole-3-thiol) and explored its potential as a flexible precursor to yield high yields of the target bis(triazolo[3,4-b][1,3,4]thiadiazines and bis(triazolo[3,4-b][1,3,4]thiadiazoles). The product structures were validated using spectrum data and elemental analysis. According to the DPPH experiment findings, the newly synthesized compounds displayed promising antioxidant properties. Compounds **6b** and **7** showed strong antioxidant activity with low  $IC_{50}$  values, indicating that these compounds might be especially effective as therapeutic agents in preventing or delaying the course of aging and age-related oxidative stress-related degenerative disorders.

## Experimental Section

**General.** Melting points were determined in open glass capillaries with a Gallenkamp apparatus and were not corrected. Infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP3-300 and Shimadzu FTIR Spirit spectrophotometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined on a Varian Mercury VX 300 NMR spectrometer using TMS as an internal standard and  $\text{DMSO-}d_6$  as a solvent. Mass spectra were measured on a GC MS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. Antioxidant activity was investigated at the Department of Microbial Genetic Resources, National Gene Bank, Agricultural Research Center (ARC), Giza, Egypt.

**Synthesis of 5,5'-(Thiobis(methylene))bis(4-amino-4H-1,2,4-triazole-3-thiol) (3).** A mixture of 2,2'-thiodiacetic acid (**1**) (0.1 mol) and thiocarbohydrazide (**2**) (0.2 mol) was warmed carefully until melting occurred and then it was kept at 170 °C for 30 min. The reaction mixture was then cooled and mixed with water (50 ml). The

precipitate was collected, washed with water and hot ethanol, and finally recrystallized from DMF/water to give **3**

**5,5'-(Thiobis(methylene))bis(4-amino-4H-1,2,4-triazole-3-thiol) (3)**. Colorless powder (80% Yield), mp. 265-267 °C; IR (cm<sup>-1</sup>): 3293, 3170 (NH<sub>2</sub>), 2568 (SH); <sup>1</sup>H-NMR (DMSO): δ 3.83 (s, 4H, SCH<sub>2</sub>), 5.52 (s, 4H, NH<sub>2</sub>), 13.60 (s, 2H, SH); <sup>13</sup>C-NMR: δ 31.8, 155.3, 168.0; MS: *m/z* (%) 290 (M<sup>+</sup>). Anal. calcd. For C<sub>6</sub>H<sub>10</sub>N<sub>8</sub>S<sub>3</sub>: C, 24.82; H, 3.47; N, 38.59; S, 33.12. Found: C, 24.95; H, 3.44; N, 38.54; S, 33.11%.

**Synthesis of 5,5'-(thiobis(methylene))bis(4-(arylideneamino)-4H-1,2,4-triazole-3-thiols) 6a-c and 7**. To a solution of the appropriate aromatic aldehydes **4a-c** and **5** (2 mmol) in glacial acetic acid (10 mL), 1 mmol of the (4-amino-4H-1,2,4-triazole-3-thiol) **3** was added and the reaction mixture was heated at reflux for 4-6 hours. The reaction mixture was filtered on hot and washed several times with hot ethanol and then recrystallized from DMF to afford the corresponding bis(Schiff bases) **6a-c** and **7**.

**5,5'-(Thiobis(methylene))bis(4-((-4-methoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol) (6a)**. Creamy powder (77% Yield), mp. 242-244 °C; IR (cm<sup>-1</sup>): 2556 (SH), 1603 (C=N); <sup>1</sup>H-NMR (DMSO): δ 3.79 (s, 6H, OCH<sub>3</sub>), 3.91 (s, 4H, SCH<sub>2</sub>), 7.03 (d, *J* = 8.5 Hz, 4H, ArH), 7.76 (d, *J* = 8.5 Hz, 4H, ArH), 9.73 (s, 2H, HC=N), 13.90 (s, 2H, SH); <sup>13</sup>C-NMR: δ 24.0, 55.4, 127.7, 128.4, 129.1, 146.5, 149.6, 150.0, 153.4; MS: *m/z* (%) 526 (M<sup>+</sup>). Anal. calcd. For C<sub>22</sub>H<sub>22</sub>N<sub>8</sub>O<sub>2</sub>S<sub>3</sub>: C, 50.17; H, 4.21; N, 21.28; S, 18.26. Found: C, 50.07; H, 4.31; N, 21.23; S, 18.23%.

**5,5'-(Thiobis(methylene))bis(4-((-4-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol) (6b)**. Yellow powder (84% Yield), mp. 248-250 °C; IR (cm<sup>-1</sup>): 2540 (SH), 1590 (C=N); <sup>1</sup>H-NMR (DMSO): δ 4.04 (s, 4H, SCH<sub>2</sub>), 8.09 (d, *J* = 9 Hz, 4H, ArH), 8.31 (d, *J* = 9 Hz, 4H, ArH), 10.45 (s, 2H, HC=N), 14.04 (s, 2H, SH); <sup>13</sup>C-NMR: δ 26.9, 124.0, 129.7, 138.1, 146.7, 148.5, 154.0, 157.6; MS: *m/z* (%) 556 (M<sup>+</sup>); Anal. calcd. For C<sub>20</sub>H<sub>16</sub>N<sub>10</sub>O<sub>4</sub>S<sub>3</sub>: C, 43.16; H, 2.90; N, 25.17; S, 17.28. Found: C, 43.31; H, 2.75; N, 25.35; S, 17.26%.

**5,5'-(Thiobis(methylene))bis(4-((-4-(dimethylamino)benzylidene)amino)-4H-1,2,4-triazole-3-thiol) (6c)**. Pale yellow powder (81% Yield), mp. 255-257 °C; IR (cm<sup>-1</sup>): 2549 (SH), 1588 (C=N); <sup>1</sup>H NMR (DMSO): δ 3.02 (s, 12H, N(CH<sub>3</sub>)<sub>2</sub>), 3.92 (s, 4H, SCH<sub>2</sub>), 6.76 (d, *J* = 9 Hz, 4H, ArH), 7.62 (d, *J* = 9 Hz, 4H, ArH), 9.44 (s, 2H, HC=N), 13.78 (s, 2H, SH); <sup>13</sup>C-NMR: δ 23.6, 42.6, 111.7, 122.7, 127.9, 145.8, 148.5, 148.9, 158.3; MS: *m/z* (%) 552 (M<sup>+</sup>). Anal. calcd. For C<sub>24</sub>H<sub>28</sub>N<sub>10</sub>S<sub>3</sub>: C, 52.15; H, 5.11; N, 25.34; S, 17.40. Found: C, 52.03; H, 5.15; N, 25.38; S, 17.37%.

**5,5'-(Thiobis(methylene))bis(4-(2-methoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol) (6d)**. Pale yellow powder (77% Yield), mp. 234-236 °C; IR (cm<sup>-1</sup>): 2543 (SH), 1579 (C=N); <sup>1</sup>H NMR (DMSO): δ 3.87 (s, 6H, OCH<sub>3</sub>), 3.97 (s, 4H, SCH<sub>2</sub>), 7.03-7.17 (m, 4H, ArH), 7.53-7.93 (m, 4H, ArH), 10.39 (s, 2H, HC=N), 13.90 (s, 2H, SH); MS: *m/z* (%) 526 (M<sup>+</sup>). Anal. calcd. For C<sub>22</sub>H<sub>22</sub>N<sub>8</sub>O<sub>2</sub>S<sub>3</sub>: C, 50.17; H, 4.21; N, 21.28; S, 18.26. Found: C, 50.27; H, 4.41; N, 21.30; S, 18.40%.

**5,5'-(Thiobis(methylene))bis(4-((-thiophen-2-ylmethylene)amino)-4H-1,2,4-triazole-3-thiol) (7a)**. Creamy powder (78% Yield), mp. 236-238 °C; IR (cm<sup>-1</sup>): 2523 (SH), 1593 (C=N); <sup>1</sup>H NMR (DMSO): δ 3.93 (s, 4H, SCH<sub>2</sub>), 7.23-7.26 (m, 2H, thiophene-4-H), 7.76 (d, *J* = 3 Hz, 2H, thiophene-3-H), 7.91 (d, *J* = 3 Hz, 2H, thiophene-5-H), 10.13 (s, 2H, HC=N), 13.92 (s, 2H, SH); <sup>13</sup>C-NMR: δ 23.8, 124.3, 126.0, 131.1, 138.0, 153.2, 156.5, 161.6; MS: *m/z* (%) 477 (M<sup>+</sup>). Anal. calcd. For C<sub>16</sub>H<sub>14</sub>N<sub>8</sub>S<sub>5</sub>: C, 40.15; H, 2.95; N, 23.41; S, 33.49. Found: C, 40.28; H, 2.98; N, 23.49; S, 33.49%.

**5,5'-(Thiobis(methylene))bis(4-(pyridin-3-ylmethylene)amino)-4H-1,2,4-triazole-3-thiol) (7b)**. Yellow powder (74% Yield), mp. 231-233 °C; IR (cm<sup>-1</sup>): 2515 (SH), 1588 (C=N); <sup>1</sup>H NMR (DMSO): δ 4.02 (s, 4H, SCH<sub>2</sub>), 7.52-8.74 (m, 6H, ArH), 8.98 (s, 2H, pyridine-2-H), 10.27 (s, 2H, HC=N), 13.99 (s, 2H, SH); MS: *m/z* (%) 468 (M<sup>+</sup>). Anal. calcd. For C<sub>18</sub>H<sub>16</sub>N<sub>10</sub>S<sub>3</sub>: C, 46.14; H, 3.44; N, 29.89; S, 20.53. Found: C, 45.90; H, 3.31; N, 30.23; S, 20.45%.

**Synthesis of bis((7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes 10a,b, 11, 14 and 15**. To a solution of the appropriate 2-bromo-ethanones **8a,b,9, 12**, and **13** (2 mmol) in a mixture of EtOH/DMF (20 mL, 5:2) containing TEA (0.1 mL), was added 5,5'-(thiobis(methylene))bis (4-amino-4H-1,2,4-triazole-3-thiol) (1

mmol) (**3**). The reaction mixture was heated at reflux for 4-6 hours. The reaction mixture was then left to cool, and the formed solid product was filtered off and crystallized from ethanol/DMF to obtain the corresponding products **10a,b**, **11**, **14**, and **15**.

**Bis((6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (10a)**. Pink powder (88% Yield), mp. 238-240 °C; IR (cm<sup>-1</sup>): 1603 (C=N); <sup>1</sup>H-NMR (DMSO): δ 4.18 (s, 4H, thiadiazine-6-H), 4.40 (s, 4H, SCH<sub>2</sub>), 7.51-7.60 (m, 6H, ArH), 7.99-8.02 (m, 4H, ArH); <sup>13</sup>C-NMR: δ 20.3, 31.6, 127.8, 128.3, 131.7, 134.1, 153.1, 157.3, 166.3; MS: *m/z* (%) 490 (M<sup>+</sup>). Anal. calcd. For C<sub>22</sub>H<sub>18</sub>N<sub>8</sub>S<sub>3</sub>: C, 53.86; H, 3.70; N, 22.84; S, 19.60. Found: C, 53.76; H, 3.80; N, 22.90; S, 19.58%.

**Bis((6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (10b)**. Buff powder (80% Yield), mp. 248-250 °C; IR (cm<sup>-1</sup>): 1588 (C=N); <sup>1</sup>H-NMR (DMSO): δ 4.16 (s, 4H, thiadiazine-6-H), 4.38 (s, 4H, SCH<sub>2</sub>), 7.60 (d, *J* = 9 Hz, 4H, ArH), 8.01 (d, *J* = 9 Hz, 4H, ArH); <sup>13</sup>C-NMR: δ 23.9, 34.2, 122.0, 124.8, 129.0, 135.3, 144.3, 150.9, 164.3; MS: *m/z* (%) 558 (M<sup>+</sup>). Anal. calcd. For C<sub>22</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>8</sub>S<sub>3</sub>: C, 47.23; H, 2.88; N, 20.03; S, 17.19. Found: C, 48.66; H, 2.76; N, 19.90; S, 17.19%.

**Bis((6-(naphthalen-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (11)**. Brown powder (82% Yield), mp. 214-216 °C; IR (cm<sup>-1</sup>): 1567 (C=N); <sup>1</sup>H NMR (DMSO): δ 4.26 (s, 4H, thiadiazine-6-H), 4.53 (s, 4H, SCH<sub>2</sub>), 7.62-7.64 (m, 4H, ArH), 7.96-8.02 (m, 6H, ArH), 8.16 (d, *J* = 9 Hz, 2H, naphthalene-3-H), 8.61 (s, 2H, naphthalene-1-H); <sup>13</sup>C-NMR: δ 23.9, 34.2, 124.8, 127.1, 127.7, 129.0, 135.4, 138.7, 139.2, 141.0, 147.8, 153.8, 159.3, 168.1; MS: *m/z* (%) 590 (M<sup>+</sup>). Anal. calcd. For C<sub>30</sub>H<sub>22</sub>N<sub>8</sub>S<sub>3</sub>: C, 61.00; H, 3.75; N, 18.97; S, 16.28. Found: C, 60.88; H, 3.81; N, 19.03; S, 16.23%.

**Bis((6-(5-bromothiophen-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (14)**. Beige powder (73% Yield), mp. >300 °C; IR (cm<sup>-1</sup>): 1574 (C=N); <sup>1</sup>H-NMR (DMSO): δ 4.10 (s, 4H, thiadiazine-6-H), 4.40 (s, 4H, SCH<sub>2</sub>), 7.41 (d, *J* = 3 Hz, 2H, thiophene-3-H), 7.74 (d, *J* = 3 Hz, 2H, thiophene-4-H); <sup>13</sup>C-NMR: δ 24.4, 35.1, 116.2, 124.8, 127.7, 129.1, 147.7, 155.7, 164.8; MS: *m/z* (%) 657 (M<sup>+</sup>). Anal. calcd. For C<sub>18</sub>H<sub>12</sub>Br<sub>2</sub>N<sub>8</sub>S<sub>3</sub>: C, 32.73; H, 1.83; N, 16.97; S, 24.27. Found: C, 32.50; H, 1.96; N, 16.86; S, 24.24%.

**Bis((6-(benzo[d]thiazol-2-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (15)**. Pale yellow powder (77% Yield), mp. 200-202 °C (decomp.); IR (cm<sup>-1</sup>): 1594 (C=N); <sup>1</sup>H-NMR (DMSO): δ 4.25 (s, 4H, thiadiazin-6-H), 4.52 (s, 4H, SCH<sub>2</sub>), 7.61-7.63 (m, 4H, benzothiazol-5,6-H), 7.97 (d, *J* = 9 Hz, 2H, benzothiazol-4-H), 8.16 (d, *J* = 9 Hz, 2H, benzothiazol-7-H); <sup>13</sup>C-NMR: δ 23.6, 34.0, 120.0, 124.8, 127.7, 129.1, 135.5, 150.1, 158.2, 158.7, 159.2, 167.6; MS: *m/z* (%) 604 (M<sup>+</sup>). Anal. calcd. For C<sub>24</sub>H<sub>16</sub>N<sub>10</sub>S<sub>5</sub>: C, 47.67; H, 2.67; N, 23.16; S, 26.51. Found: C, 47.51; H, 2.73; N, 23.21; S, 26.50%.

**Synthesis of bis((7-(2-arylhydrazono)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfanes 17a-d**. To a solution of 5,5'-(thiobis(methylene))bis(4-amino-4H-1,2,4-triazole-3-thiol) (**3**) (1 mmol) in boiling DMF (2 mL), 2-oxo-N'-phenylpropanehydrazonoyl chlorides (2 mmol) **16a-d** in absolute ethanol (20 mL) containing TEA (0.1 mL), was added. The reaction mixture was heated at refluxed for 6-8 hours. The formed solid products were filtered off to get corresponding products **17a-d**.

**Bis((6-methyl-7-(2-phenylhydrazineylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (17a)**. Olive green powder (79% Yield), mp. 260-262 °C; IR (cm<sup>-1</sup>): 3178 (NH), 1598 (C=N); <sup>1</sup>H-NMR (DMSO): δ 2.46 (s, 6H, CH<sub>3</sub>), 4.14 (s, 4H, SCH<sub>2</sub>), 6.96-7.34 (m, 10H, ArH), 10.23 (s, 2H, NH); <sup>13</sup>C-NMR: δ 13.7, 24.3, 114.2, 121.7, 131.8, 141.0, 147.7, 150.1, 154.6, 158.0; MS: *m/z* (%) 574 (M<sup>+</sup>). Anal. calcd. For C<sub>24</sub>H<sub>22</sub>N<sub>12</sub>S<sub>3</sub>: C, 50.16; H, 3.86; N, 29.25; S, 16.74. Found: C, 50.35; H, 3.79; N, 29.14; S, 16.71%.

**Bis((6-methyl-7-(2-(p-tolyl)hydrazineylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (17b)**. Green powder (78% Yield), mp. 266-268 °C; IR (cm<sup>-1</sup>): 3216 (NH) 1579 (C=N); <sup>1</sup>H-NMR (DMSO): δ 2.25 (s, 6H, CH<sub>3</sub>), 2.44 (s, 6H, CH<sub>3</sub>), 4.12 (s, 4H, SCH<sub>2</sub>), 7.11 (d, *J* = 9 Hz, 4H, ArH), 7.23 (d, *J* = 9 Hz, 4H, ArH), 10.14 (s, 2H,

NH);  $^{13}\text{C}$ -NMR:  $\delta$  20.3, 20.9, 23.1, 114.4, 116.0, 129.6, 131.1, 136.6, 141.3, 150.7, 156.6; MS:  $m/z$  (%) 602 ( $\text{M}^+$ ). Anal. calcd. For  $\text{C}_{26}\text{H}_{26}\text{N}_{12}\text{S}_3$ : C, 51.81; H, 4.35; N, 27.89; S, 15.96. Found: C, 51.92; H, 4.41; N, 27.93; S, 15.96%.

**Bis((7-(2-(4-methoxyphenyl)hydrazineylidene)-6-methyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (17c)**. Dark green powder (75% Yield), mp. 270 °C; IR ( $\text{cm}^{-1}$ ): 3225 (NH), 1591 (C=N);  $^1\text{H}$ -NMR (DMSO):  $\delta$  2.43 (s, 6H,  $\text{CH}_3$ ), 3.73 (s, 6H,  $\text{OCH}_3$ ), 4.12 (s, 4H,  $\text{SCH}_2$ ), 6.90 (d,  $J = 9$  Hz, 4H, ArH), 7.27 (d,  $J = 9$  Hz, 4H, ArH), 10.11 (s, 2H, NH);  $^{13}\text{C}$ -NMR:  $\delta$  17.2, 24.0, 59.3, 114.5, 120.6, 129.5, 145.8, 152.3, 157.9, 160.5, 161.3; MS:  $m/z$  (%) 634 ( $\text{M}^+$ ). Anal. calcd. For  $\text{C}_{26}\text{H}_{26}\text{N}_{12}\text{O}_2\text{S}_3$ : C, 49.20; H, 4.13; N, 26.48; S, 15.15. Found: C, 49.35; H, 4.03; N, 26.43; S, 15.17%.

**Bis((6-methyl-7-(2-(4-nitrophenyl)hydrazineylidene)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methyl)sulfane (17d)**. Dark green powder (73% Yield), mp. > 300 °C; IR ( $\text{cm}^{-1}$ ): 3188 (NH), 1594 (C=N);  $^1\text{H}$ -NMR (DMSO):  $\delta$  2.46 (s, 6H,  $\text{CH}_3$ ), 4.12 (s, 4H,  $\text{SCH}_2$ ), 7.41 (d,  $J = 9$  Hz, 4H, ArH), 8.13 (d,  $J = 9$  Hz, 4H, ArH), 10.88 (s, 2H, NH); MS:  $m/z$  (%) 664 ( $\text{M}^+$ ). Anal. calcd. For  $\text{C}_{24}\text{H}_{20}\text{N}_{14}\text{O}_4\text{S}_3$ : C, 43.37; H, 3.03; N, 29.50; S, 14.47. Found: C, 43.25; H, 3.14; N, 29.54; S, 14.46%.

**Synthesis of synthesis of bis([1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylmethyl)sulfanes 20 and 21.** To a solution of 5,5'-(thiobis(methylene))bis(4-amino-4H-1,2,4-triazole-3-thiol) (**3**) (1 mmol) in phosphorous oxychloride (10 mL), carboxylic acids **18** or **19** (2 mmol) was added. The reaction mixture was heated at refluxed for 5-7 hours. After cooling, the reaction mixture was then poured over crushed ice. The precipitated products were filtered and recrystallized from ethanol to give the corresponding products **20** and **21**.

**Bis((6-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)sulfane (20)**. Creamy powder (78% Yield), mp. 160 °C; IR ( $\text{cm}^{-1}$ ): 1607 (C=N);  $^1\text{H}$ -NMR (DMSO):  $\delta$  3.84 (s, 6H,  $\text{OCH}_3$ ), 4.38 (s, 4H,  $\text{SCH}_2$ ), 7.06 (d,  $J = 9$  Hz, 4H, ArH), 7.79 (d,  $J = 9$  Hz, 4H, ArH);  $^{13}\text{C}$ -NMR:  $\delta$  23.9, 56.2, 114.7, 120.3, 129.3, 152.4, 161.1, 162.3, 169.9; MS:  $m/z$  (%) 522 ( $\text{M}^+$ ). Anal. calcd. For  $\text{C}_{22}\text{H}_{18}\text{N}_8\text{O}_2\text{S}_3$ : C, 50.56; H, 3.47; N, 21.44; S, 18.40. Found: C, 50.73; H, 3.23; N, 21.52; S, 18.41%.

**Bis((6-(thiophen-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl)sulfane (21)**. Dark beige powder (69% Yield), mp. 226-228 °C; IR ( $\text{cm}^{-1}$ ): 1558 (C=N);  $^1\text{H}$ -NMR (DMSO):  $\delta$  4.35 (s, 4H,  $\text{SCH}_2$ ), 7.24-7.27 (m, 2H, thiophene-4-H), 7.86 (d,  $J = 3$  Hz, 2H, thiol-3-H), 7.95 (d,  $J = 3$  Hz, 2H, thiophene-5-H);  $^{13}\text{C}$ -NMR:  $\delta$  25.5, 126.7, 127.6, 128.0, 129.1, 156.3, 158.6, 167.7; MS:  $m/z$  (%) 473 ( $\text{M}^+$ ); Anal. calcd. For  $\text{C}_{16}\text{H}_{10}\text{N}_8\text{S}_5$  (473.96): C, 40.49; H, 2.12; N, 23.61; S, 33.77. Found: C, 40.40; H, 2.15; N, 23.64; S, 33.73%.

## Material and methods

**Antioxidant activity.** The antioxidant activity of fifteen synthesized compounds was investigated utilizing DPPH scavenging<sup>54,55</sup>. The compounds were dissolved in DMSO at a concentration of 1000  $\mu\text{g}/\text{ml}$ . DPPH (2,2-diphenyl-1-hydrazine) is produced in methanol at 0.004% and stored in the dark at 4 °C. The reaction mixture was created by adding 20  $\mu\text{L}$  of dissolved testing compounds to 180  $\mu\text{L}$  of DPPH on 96 well plates (flat bottom, Greiner bio). All compounds were tested in triplicate, and positive control was performed for each compound to eliminate the effect of compound color from calculations. The initial screening was performed in the reaction mixture of 96 well plants at a single compound concentration of 1 mg/ml to identify their antioxidant (to reach the final concentration of 100  $\mu\text{g}/\text{ml}$ ). Using a microplate reader (Tecan Infinite 200 Pro), the residual DPPH was measured at 520 nm. The percentage of antioxidant activity (% AA) was calculated using the following equation:  

$$\% \text{ inhibition} = [(\text{OD}(540) (\text{Blank}) - \text{OD}(540) (\text{Sample})) / \text{OD}(540) (\text{Blank})] \times 100$$

## Statistical analysis

The GraphPad Prism® software package (version 8) was used to process the data.

**Table 1.** Calculated IC<sub>50</sub> values of tested compounds for their antioxidant activity

Tested compounds	IC <sub>50</sub> (µg/ml)	Goodness of fit (R <sup>2</sup> )
<b>7</b>	9.4 ± 1.4	0.979
<b>6b</b>	14.7 ± 0.4	0.995
<b>6a</b>	25.1 ± 0.1	0.976
<b>6c</b>	35.6 ± 1.5	0.995
<b>17c</b>	46.4 ± 6.9	0.938
<b>15</b>	116.9 ± 6.6	0.990
<b>17a</b>	143.9 ± 2.3	0.965
<b>17d</b>	188.4 ± 12.6	0.964
<b>17b</b>	189.5 ± 14.5	0.927
<b>Quercetin dihydrate</b>	7.64 ± 0.9	0.967

## Supplementary Material

Copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra of synthesized compounds are available in the supplementary material file associated with this manuscript.

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