A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2024** (vii) 202412181

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

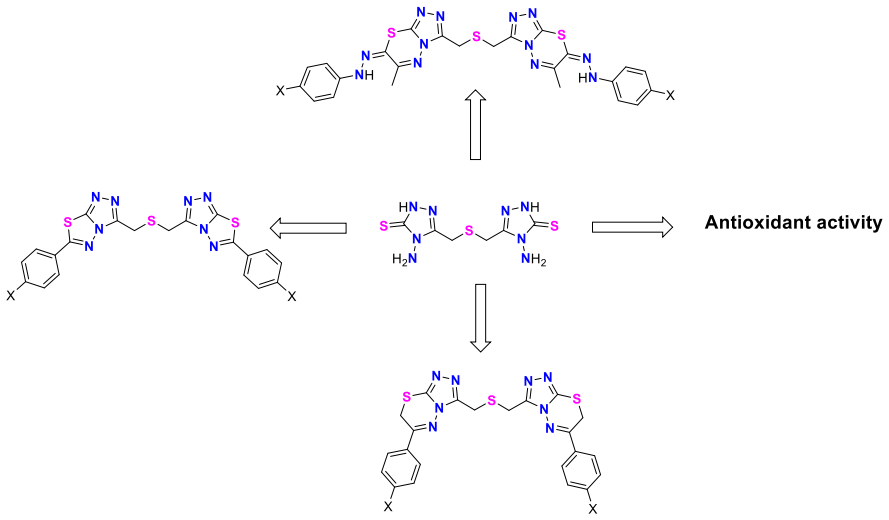
Ahmed H. M. Elwahy[a]*, Ahmed R. S. Ginidi[a], Mohamed R. Shaaban[b], Akram H. Mohamed[c], Hatem M. Gaber[d], Laila I. Ibrahim[d], Ahmed M. Farag[a], and Mostafa E. Salem[e]*

[a] Department of Chemistry, Faculty of Science, Cairo University, Giza, Egyp; [b] Department of Chemistry, Faculty of Applied Science, Umm Al-Qura University, Makkah Almukaramah, Saudi Arabia; [c] Department of Microbial Genetic Resources, National Gene Bank, Agricultural Research Center (ARC), Giza, Egypt [d] National Organization for Drug Control and Research, P.O. Box 29, Cairo, Egypt [e] Department of Chemistry, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), P.O. Box 90950, Riyadh 11623, Saudi Arabia
E-mail: aelwahy@hotmail.com, aelwahy@cu.edu.eg, m_chem788@yahoo.com, meaSalem@imamu.edu.sa

Received mm-dd-yyyy **Accepted Manuscript** mm-dd-yyyy **Published on line** mm-dd-yyyy

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

Cite as *Arkivoc* **2024** (7) 202412181
DOI: https://doi.org/10.24820/ark.5550190.p012.181

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.[1–3] 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.[4–6] Among several synthesized compounds, derivatives of 1,2,4-triazoles [7–9], 1,3,4-thiadiazoles [10], and 1,3,4-thiadiazoles[11,12] 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 [13–15]. 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 [16–18]. They could be prepared from 4-amino-1,2,4-triazole-3-thiol utilizing the nucleophilic character of its amino and mercapto groups. [19,20]

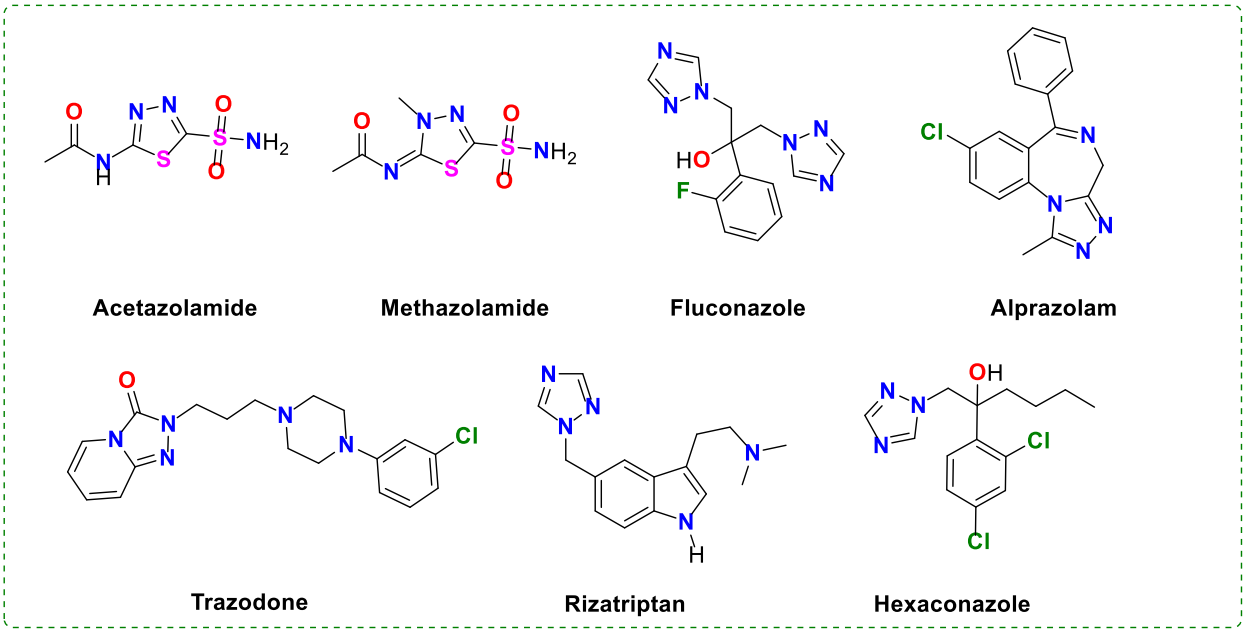
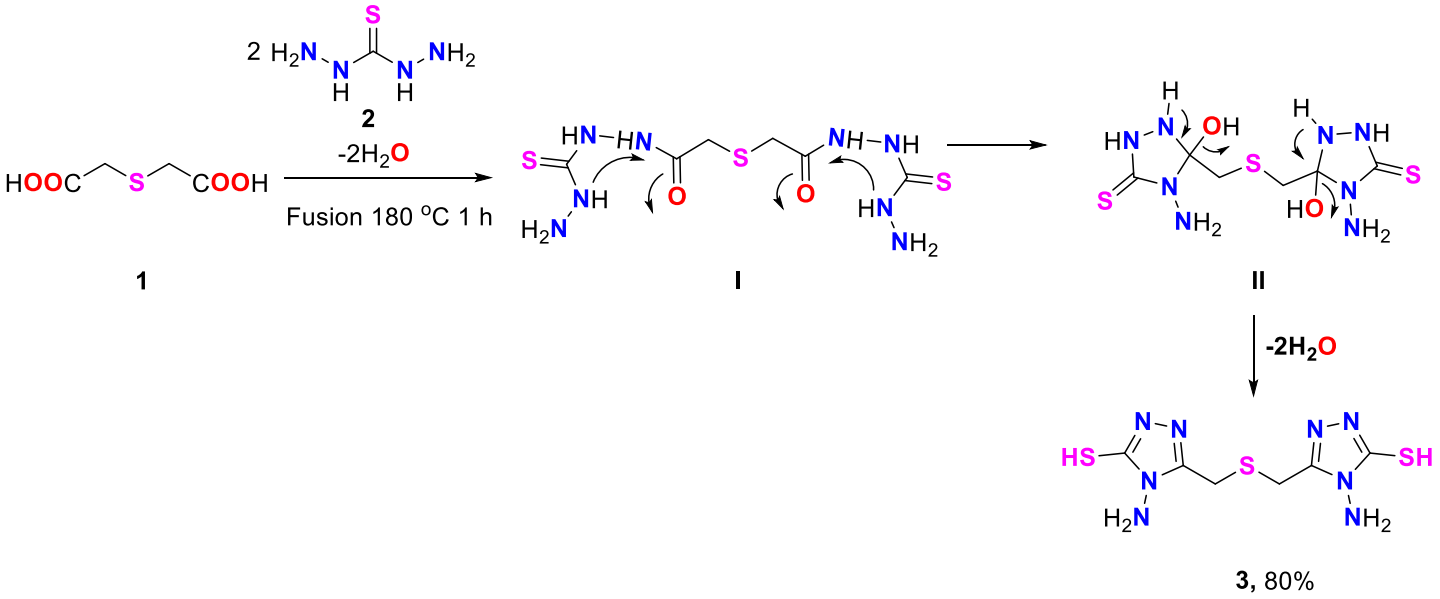


Figure 1 Some drugs containing 1,2,4-triazole and 1,3,4-thiadiazole moieties

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

Results and Discussion

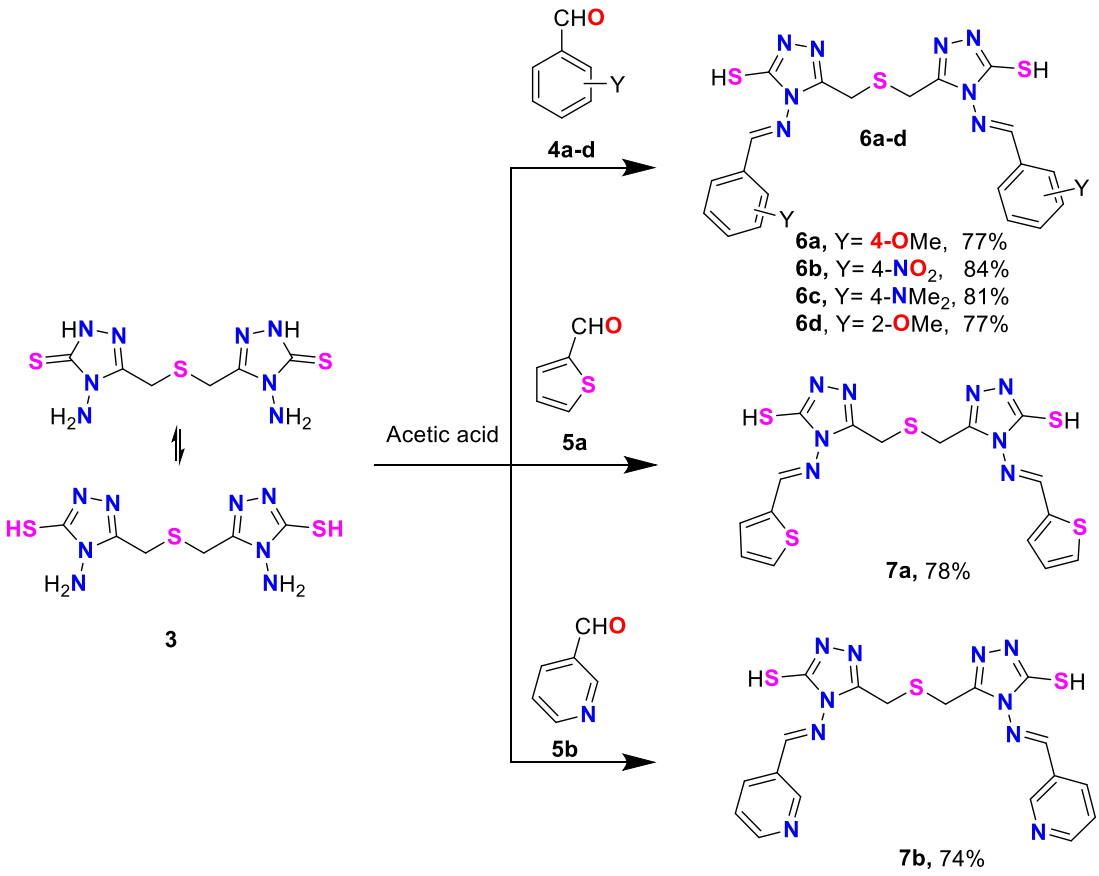
Bis(4-amino-4*H*-1,2,4-triazole-3-thiol) (**3**) was selected as a versatile precursor for the desired fused triazoles. It produced in an 80% yield by reacting one mole of 2,2'-thiodiacetic acid **1** with two moles of thiocarbohydrazide (**2**) under fusion conditions. The reaction begins with the elimination 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 cm^{-1} were identified as amine groups. Characteristic singlet signals for the SCH_2 and NH_2 protons were detected in its ^{1}H-NMR spectrum in DMSO-d_6 solution at 3.83, and 5.52 ppm, respectively. It also revealed a characteristic signal at δ 13.60 indicating the preference of the thiol over the thione form.[48]

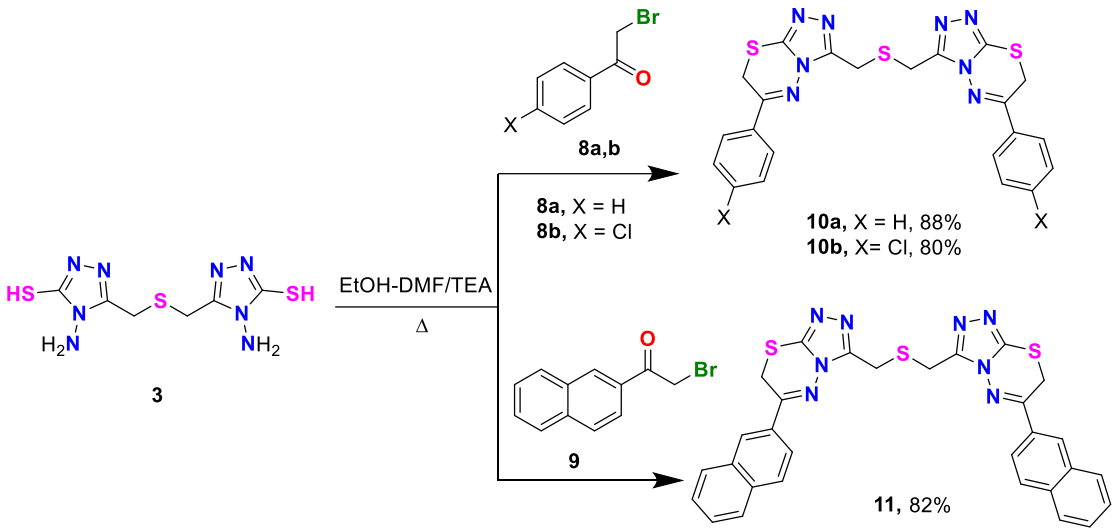
The synthetic utility of the bis-amine **3** as a building unit for a variety of heterocyclic systems was studied according to previous investigations.[49,50] The reactivity of bis-amine **3** to the production of new Schiff bases was first explored. Schiff base ligands are easily synthesized by the 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.[51,52] 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)-4*H*-1,2,4-triazole-3-thiols) **6a-d** and **7a,b**

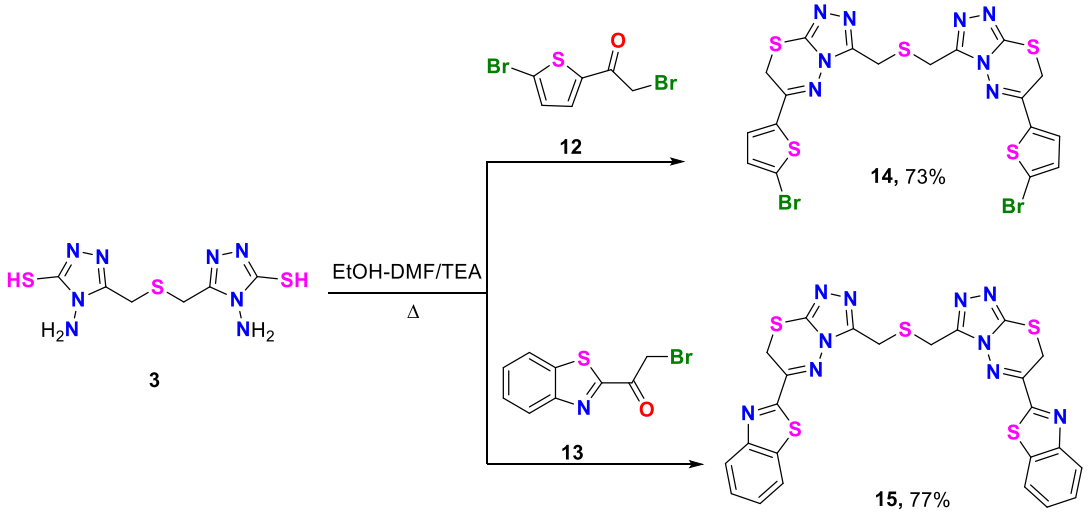
The reaction of NH_2 with the -CHO groups of the aldehydes, resulting in the formation of the corresponding Schiff bases, was confirmed by the disappearance of the NH_2 absorption bands in both the IR and ^{1}H-NMR spectra of **6a**. The ^{1}H-NMR spectrum of compound **6a** showed singlet signals at 3.79, 3.91, and 13.90, which belonged to the -OCH_3, -SCH_2, and -SH groups, respectively. In addition to the typical benzylidene signal at δ 9.73, the ^{1}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((7*H*-[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).

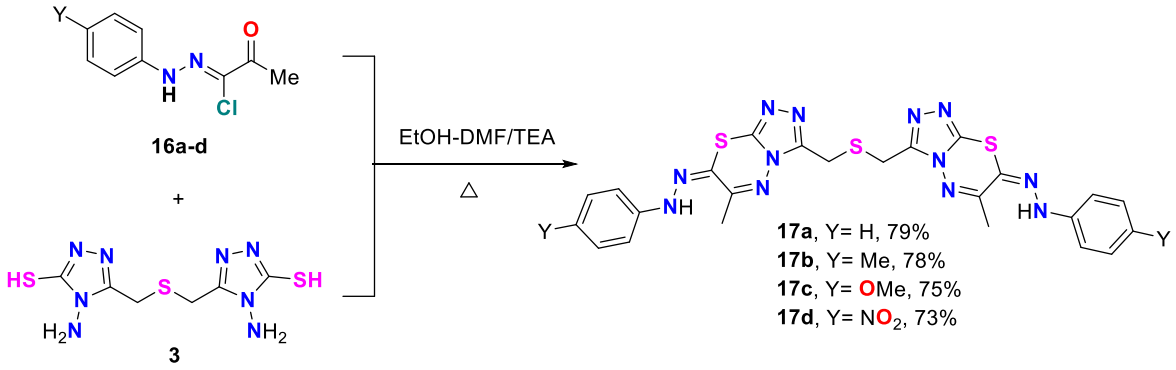


Scheme 4. Synthesis of bis((7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes **14** and **15**

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_2 stretching bands in the IR spectrum of triazolothiadiazine **14** and the lack of the main amine signal in their 1H-NMR spectra supported the cyclocondensation of the suitable α-bromoketone **12** with bis-aminotriazole **3**. The existence of thiadiazine ring SCH_2 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_2 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).

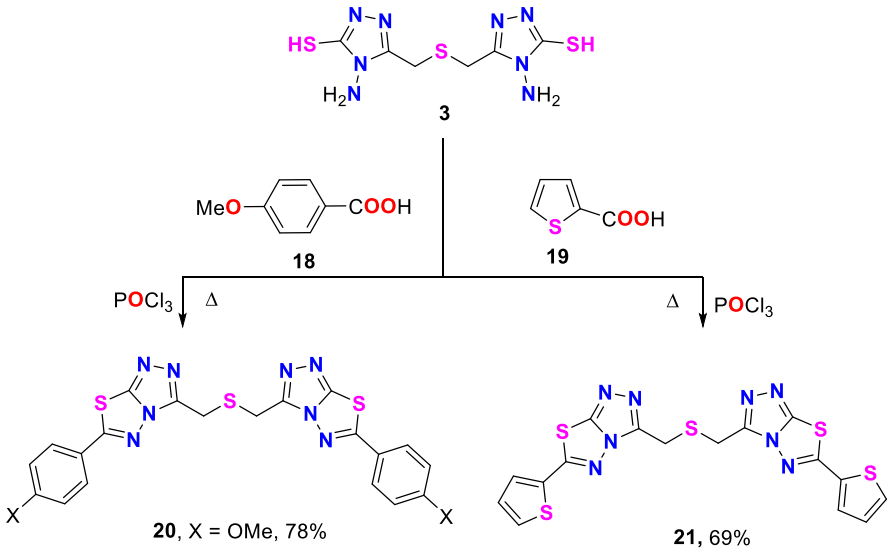
In refluxing EtOH containing triethylamine as a base, compound **3** was allowed to react with 2-oxo-N'-phenylpropanehydrazonoyl chloride **16a-d**. Reflux was carried out for 4-8 hours (monitored by thin-layer chromatography (TLC)) to yield bis(([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)sulfanes **17a-d** in 73–79%

yields (Scheme 5). The structures of these products were determined using elemental analysis and spectral data. The ^{1}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 -SCH_2 and CH_3 groups. The IR spectra revealed one band at 3188 cm^{-1} assigned to the NH group and the absence of the absorption band assigned to the NH_2 group.



Scheme 5. Synthesis of bis((7-(2-arylhydrazono)-7*H*-[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-ylmethyl)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}H NMR spectrum of compound **20** also displayed a characteristic singlet signal integrating four protons at δ 4.38 and assigned to the SCH_2 of the linker. Furthermore, the IR spectrum of **20** demonstrated a lack of absorption bands corresponding to the NH_2 or 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 IC_{50} (the concentration of test sample that causes 50% quenching of the UV absorption of DPPH) calculations. According to IC50 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[53].

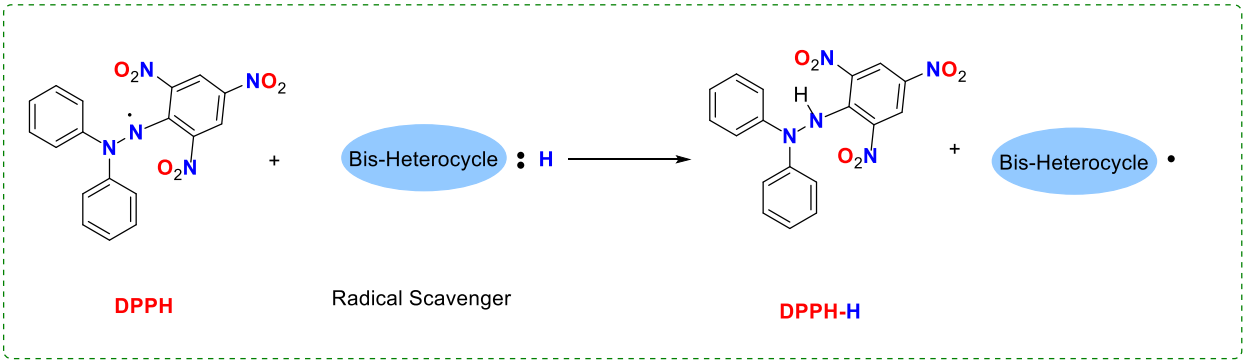


Figure 2. Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant.

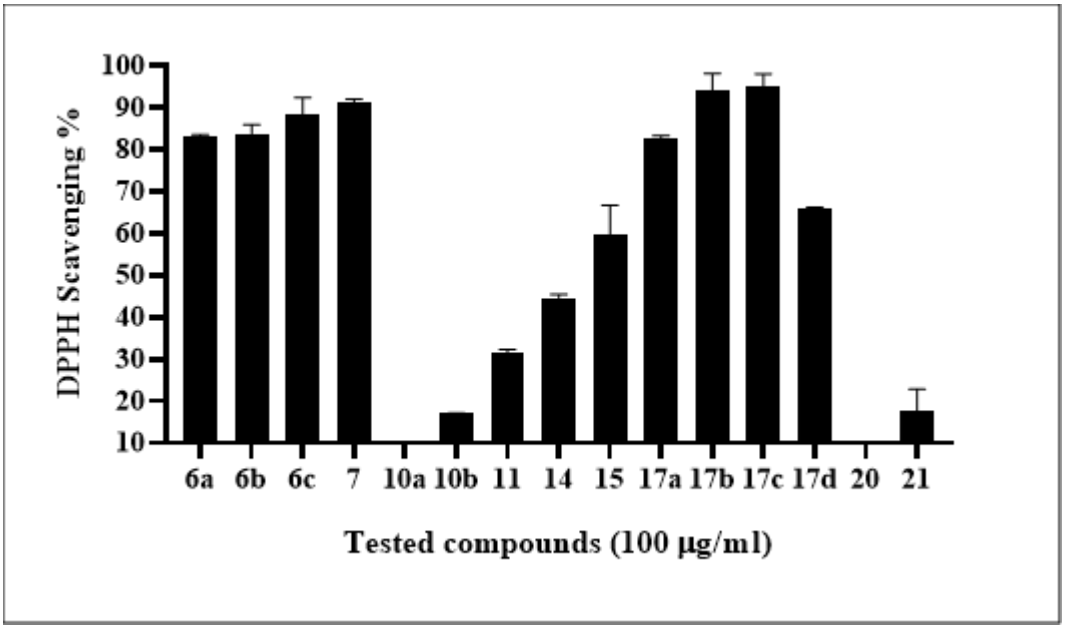


Figure 3. Primary screening of tested compounds for their antioxidant activity at a single concentration (100 µg/ml)

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 dehydrate.

Structure-activity relationship

According to the DPPH assay results, compounds (**6b** and **7**) have high antioxidant activity with low IC50 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.

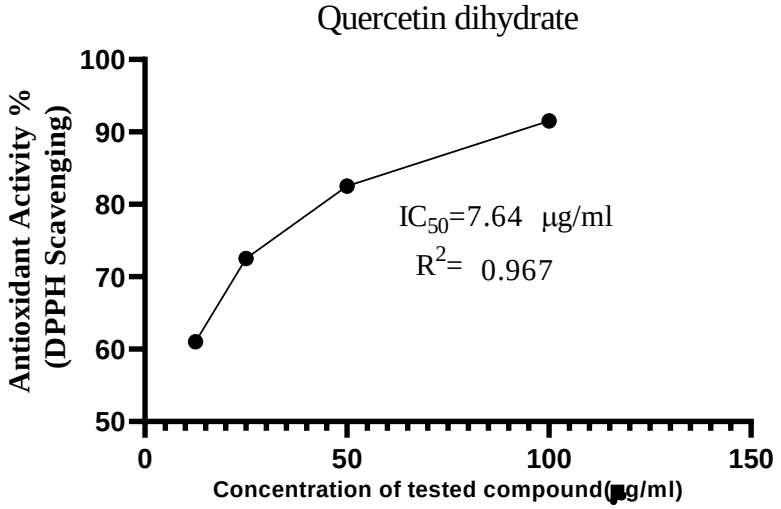
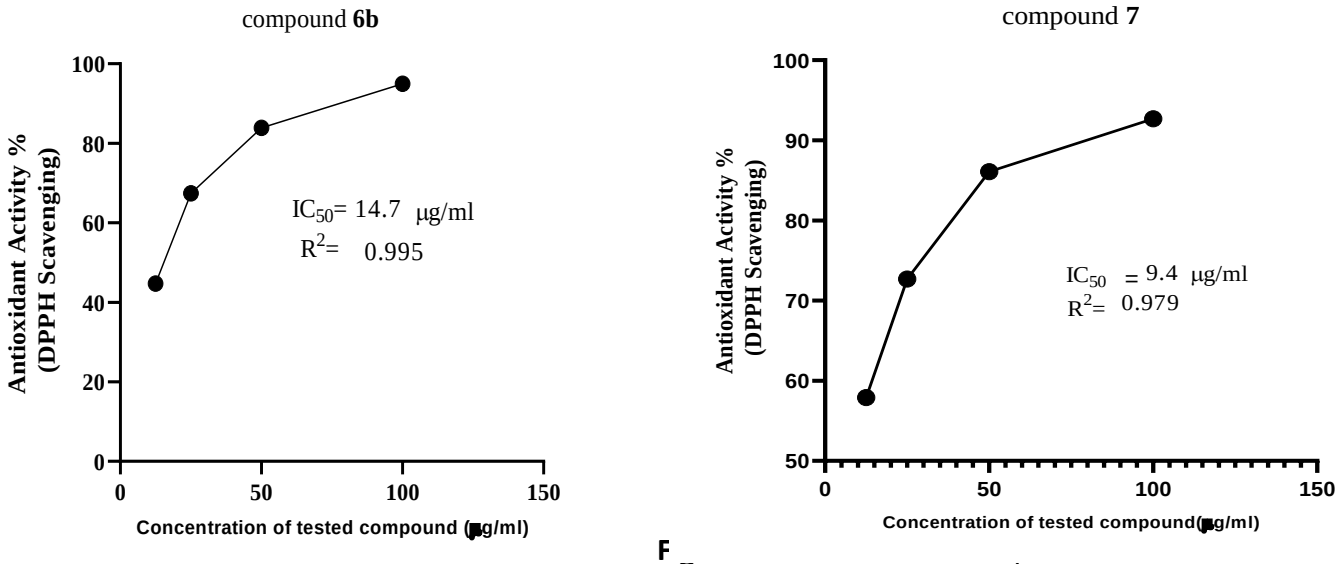


Figure 4. Concentration-response curve of DPPH scavenging by the positive control quercetin dihydrate.



F ng
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 IC50 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 PyeUnicam SP3-300 and Shimadzu FTIR Spirit spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined on a Varian Mercury VX 300NMR spectrometer using TMS as an internal standard and DMSO-*d6* 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-4*H*-1,2,4-triazole-3-thiol) (3). White powder (80% Yield), mp. 265-267 °C; IR (cm^{-1}): 3293, 3170 (NH_2), 2568 (SH); ^{1}H-NMR (DMSO): δ 3.83 (s, 4H, SCH_2), 5.52 (s, 4H, NH_2), 13.60 (s, 2H, SH); ^{13}C-NMR: δ 31.8, 155.3, 168.0; MS: *m/z* (%) 290 (M^+). Anal. calcd. For $C_6H_{10}N_8S_3$: 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)-4*H*-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-4*H*-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)-4*H*-1,2,4-triazole-3-thiol) (**6a**). Creamy powder (77% Yield), mp. 242-244 °C; IR (cm^{-1}): 2556 (SH), 1603 (C=N); ^{1}H- NMR (DMSO): δ 3.79 (s, 6H, OCH_3), 3.91 (s, 4H, SCH_2), 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); ^{13}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^+). Anal. calcd. For $C_{22}H_{22}N_8O_2S_3$: 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)-4*H*-1,2,4-triazole-3-thiol) (**6b**). Yellow powder (84% Yield), mp. 248-250 °C; IR (cm^{-1}): 2540 (SH), 1590 (C=N); ^{1}H-NMR (DMSO): δ 4.04 (s, 4H, SCH_2), 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); ^{13}C-NMR: δ 26.9, 124.0, 129.7, 138.1, 146.7, 148.5, 154.0, 157.6; MS: *m/z* (%) 556 (M^+); Anal. calcd. For $C_{20}H_{16}N_{10}O_4S_3$: 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)-4*H*-1,2,4-triazole-3-thiol) (**6c**). Pale yellow powder (81% Yield), mp. 255-257 °C; IR (cm^{-1}): 2549 (SH), 1588 (C=N); ^{1}H NMR (DMSO): δ 3.02 (s, 12H, $N(CH_3)_2$), 3.92 (s, 4H, SCH_2), 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); ^{13}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^+). Anal. calcd. For $C_{24}H_{28}N_{10}S_3$: 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)-4*H*-1,2,4-triazole-3-thiol) (**6d**). Pale yellow powder (77% Yield), mp. 234-236 °C; IR (cm^{-1}): 2543 (SH), 1579 (C=N); ^{1}H NMR (DMSO): δ 3.87 (s, 6H, OCH_3), 3.97 (s, 4H, SCH_2), 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^+). Anal. calcd. For $C_{22}H_{22}N_8O_2S_3$: 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)-4*H*-1,2,4-triazole-3-thiol) (**7a**). Creamy powder (78% Yield), mp. 236-238 °C; IR (cm^{-1}): 2523 (SH), 1593 (C=N); ^{1}H NMR (DMSO): δ 3.93 (s, 4H, SCH_2), 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); ^{13}C-NMR: δ 23.8, 124.3, 126.0, 131.1, 138.0, 153.2, 156.5, 161.6; MS: *m/z* (%) 477 (M^+). Anal. calcd. For $C_{16}H_{14}N_8S_5$: 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)-4*H*-1,2,4-triazole-3-thiol) (**7b**). Yellow powder (74% Yield), mp. 231-233 °C; IR (cm^{-1}): 2515 (SH), 1588 (C=N); ^{1}H NMR (DMSO): δ 4.02 (s, 4H, SCH_2), 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^+). Anal. calcd. For $C_{18}H_{16}N_{10}S_3$: 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((7*H*-[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-4*H*-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-7*H*-[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^{-1}): 1603 (C=N); ^{1}H-NMR (DMSO): *δ* 4.18 (s, 4H, thiadiazine-6-H), 4.40 (s, 4H, SCH_2), 7.51-7.60 (m, 6H, ArH), 7.99-8.02 (m, 4H, ArH); ^{13}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^+). Anal. calcd. For $C_{22}H_{18}N_8S_3$: 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)-7*H*-[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^{-1}): 1588 (C=N); ^{1}H-NMR (DMSO): *δ* 4.16 (s, 4H, thiadiazine-6-H), 4.38 (s, 4H, SCH_2), 7.60 (d, *J* = 9 Hz, 4H, ArH), 8.01 (d, *J* = 9 Hz, 4H, ArH); ^{13}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^+). Anal. calcd. For $C_{22}H_{16}Cl_2N_8S_3$: 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)-7*H*-[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^{-1}): 1567 (C=N); ^{1}H NMR (DMSO): *δ* 4.26 (s, 4H, thiadiazine-6-H), 4.53 (s, 4H, SCH_2), 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); ^{13}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^+). Anal. calcd. For $C_{30}H_{22}N_8S_3$: 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)-7*H*-[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^{-1}): 1574 (C=N); ^{1}H-NMR (DMSO): δ 4.10 (s, 4H, thiadiazine-6-H), 4.40 (s, 4H, SCH_2), 7.41 (d, *J* = 3 Hz, 2H, thiophene-3-H), 7.74 (d, *J* = 3 Hz, 2H, thiophene-4-H); ^{13}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^+). Anal. calcd. For $C_{18}H_{12}Br_2N_8S_3$: 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)-7*H*-[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^{-1}): 1594 (C=N); ^{1}H-NMR (DMSO): δ 4.25 (s, 4H, thiadiazin-*6-H), 4.52 (s, 4H, SCH_2), 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); ^{13}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^+). Anal. calcd. For $C_{24}H_{16}N_{10}S_5$: 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)-7*H*-[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-4*H*-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)-7*H*-[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^{-1}): 3178 (NH), 1598 (C=N); ^{1}H-NMR (DMSO): *δ* 2.46 (s, 6H, CH_3), 4.14 (s, 4H, SCH_2), 6.96-7.34 (m, 10H, ArH), 10.23 (s, 2H, NH); ^{13}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^+). Anal. calcd. For $C_{24}H_{22}N_{12}S_3$: 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)-7*H*-[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^{-1}): 3216 (NH) 1579 (C=N); ^{1}H-NMR (DMSO): *δ* 2.25 (s, 6H, CH_3), 2.44 (s, 6H, CH_3), 4.12 (s, 4H, SCH_2), 7.11 (d, *J* = 9 Hz, 4H, ArH), 7.23 (d, *J* = 9 Hz, 4H, ArH), 10.14 (s, 2H,

NH); ^{13}C-NMR: *δ* 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 (M^+). Anal. calcd. For $C_{26}H_{26}N_{12}S_3$: 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-7*H*-[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 (cm^{-1}): 3225 (NH), 1591 (C=N); ^{1}H-NMR (DMSO): *δ* 2.43 (s, 6H, CH_3), 3.73 (s, 6H, OCH_3), 4.12 (s, 4H, SCH_2), 6.90 (d, *J* = 9 Hz, 4H, ArH), 7.27 (d, *J* = 9 Hz, 4H, ArH), 10.11 (s, 2H, NH); ^{13}C-NMR: *δ* 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 (M^+). Anal. calcd. For $C_{26}H_{26}N_{12}O_2S_3$: 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)-7*H*-[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 (cm^{-1}): 3188 (NH), 1594 (C=N); ^{1}H-NMR (DMSO): *δ* 2.46 (s, 6H, CH_3), 4.12 (s, 4H, 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 (M^+) Anal. calcd. For $C_{24}H_{20}N_{14}O_4S_3$: 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-4*H*-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 (cm^{-1}): 1607 (C=N); ^{1}H-NMR (DMSO): *δ* 3.84 (s, 6H, OCH_3), 4.38 (s, 4H, SCH_2), 7.06 (d, *J* = 9 Hz, 4H, ArH), 7.79 (d, *J* = 9 Hz, 4H, ArH); ^{13}C-NMR: *δ* 23.9, 56.2, 114.7, 120.3, 129.3, 152.4, 161.1, 162.3, 169.9; MS: *m/z* (%) 522 (M^+). Anal. calcd. For $C_{22}H_{18}N_8O_2S_3$: 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 (cm^{-1}): 1558 (C=N); ^{1}H-NMR (DMSO): *δ* 4.35 (s, 4H, 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}C-NMR: *δ* 25.5, 126.7, 127.6, 128.0, 129,1, 156.3, 158.6, 167.7; MS: *m/z* (%) 473 (M^+); Anal. calcd. For $C_{16}H_{10}N_8S_5$ (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 [54,55]. The compounds were dissolved in DMSO at a concentration of 1000 µg/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 µL of dissolved testing compounds to 180 µL of DPPH on 96 well plates (flat bottom, Greinier 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 1mg/ml to identify their antioxidant (to reach the final concentration of 100 µg/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:

% inhibition = [(OD (540) (Blank) –OD (540) (Sample) / OD (540) (Blank)] x 100

Statistical analysis

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

Table 1. Calculated IC_{50} values of tested compounds for their antioxidant activity

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

Supplementary Material

Copies of ^{1}H and ^{13}C NMR spectra of synthesized compounds are available in the supplementary material file associated with this manuscript.

References

(1) de Torre, M. P.; Vizmanos, J. L.; Cavero, R. Y.; Calvo, M. I. Improvement of Antioxidant Activity of Oregano (Origanum Vulgare L.) with an Oral Pharmaceutical Form. *Biomed. Pharmacother.* **2020**, *129*, 110424.
https://doi.org/10.1016/j.biopha.2020.110424

(2) Tan, B. L.; Norhaizan, M. E.; Liew, W.-P.-P.; Sulaiman Rahman, H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Front. Pharmacol.* **2018**, *9*, 1162.
https://doi.org/10.3389/fphar.2018.01162

(3) Chaudhary, P.; Janmeda, P.; Docea, A. O.; Yeskaliyeva, B.; Abdull Razis, A. F.; Modu, B.; Calina, D.; Sharifi-Rad, J. Oxidative Stress, Free Radicals and Antioxidants: Potential Crosstalk in the Pathophysiology of Human Diseases. *Front. Chem.* **2023**, *11*, 1158198.
https://doi.org/10.3389/fchem.2023.1158198

(4) Shalaby, M. A.; Fahim, A. M.; Rizk, S. A. Antioxidant Activity of Novel Nitrogen Scaffold with Docking Investigation and Correlation of DFT Stimulation. *RSC Adv.* **2023**, *13* (21), 14580–14593. https://doi.org/10.1039/D3RA02393A

(5) Kaddouri, Y.; Abrigach, F.; Yousfi, E. B.; El Kodadi, M.; Touzani, R. New Thiazole, Pyridine and Pyrazole Derivatives as Antioxidant Candidates: Synthesis, DFT Calculations and Molecular Docking Study. *Heliyon* **2020**, *6* (1). https://doi.org/10.1016/j.heliyon.2020.e03185

(6) Javaid, M.; Haq, I.-U.; Nadeem, H.; Fatima, H.; Khan, A.-U.; Irshad, N. Design, Synthesis and Screening of Indole Acetic Acid-Based Tri-Azo Moieties as Antioxidants, Anti-Microbial and Cytotoxic Agents. *Front. Pharmacol.* **2023**, *14*, 1084181. https://doi.org/10.3389/fphar.2023.1084181

(7) Al-Mansury, S.; Balakit, A. A.; Alkazazz, F. F.; Ghaleb, R. A. Synthesis, Antiproliferative and Antioxidant Activity of 3-Mercapto-1, 2, 4-Triazole Derivatives as Combretastatin A-4 Analogues. *Pharm. Chem. J.* **2021**, *55*, 556–565. https://doi.org/10.1007/s11094-021-02459-0

(8) Saadaoui, I.; Krichen, F.; Salah, B. Ben; Mansour, R. Ben; Miled, N.; Bougatef, A.; Kossentini, M. Design, Synthesis and Biological Evaluation of Schiff Bases of 4-Amino-1, 2, 4-Triazole Derivatives as Potent Angiotensin Converting Enzyme Inhibitors and Antioxidant Activities. *J. Mol. Struct.* **2019**, *1180*, 344–354. https://doi.org/10.1016/j.molstruc.2018.12.008

(9) Pachuta-Stec, A. Antioxidant Activity of 1, 2, 4-Triazole and Its Derivatives: A Mini-Review. *Mini Rev. Med. Chem.* **2022**, *22* (7), 1081–1094. https://doi.org/10.2174/1389557521666210401091802

(10) Jakovljević, K.; Joksović, M. D.; Botta, B.; Jovanović, L. S.; Avdović, E.; Marković, Z.; Mihailović, V.; Andrić, M.; Trifunović, S.; Marković, V. Novel 1, 3, 4-Thiadiazole Conjugates Derived from Protocatechuic Acid: Synthesis, Antioxidant Activity, and Computational and Electrochemical Studies. *Comptes Rendus Chim.* **2019**, *22* (8), 585–598. https://doi.org/10.1016/j.crci.2019.06.001

(11) Gerasimova, E. L.; Gazizullina, E. G.; Igdisanova, D. I.; Sidorova, L. P.; Tseitler, T. A.; Emelianov, V. V; Chupakhin, O. N.; Ivanova, A. V. Antioxidant Properties of 2, 5-Substituted 6 H-1, 3, 4-Thiadiazines Promising for Experimental Therapy of Diabetes Mellitus. *Russ. Chem. Bull.* **2022**, *71* (12), 2730–2739. https://doi.org/10.1007/s11172-022-3702-0

(12) Čačić, M.; Pavić, V.; Molnar, M.; Šarkanj, B.; Has-Schon, E. Design and Synthesis of Some New 1, 3, 4-Thiadiazines with Coumarin Moieties and Their Antioxidative and Antifungal Activity. *Molecules* **2014**, *19* (1), 1163–1177. https://doi.org/10.3390/molecules19011163

(13) Aggarwal, R.; Sumran, G. An Insight on Medicinal Attributes of 1, 2, 4-Triazoles. *Eur. J. Med. Chem.* **2020**, *205*, 112652. https://doi.org/10.1016/j.ejmech.2020.112652

(14) Fan, Y.; Ke, X.; Liu, M. Coumarin–Triazole Hybrids and Their Biological Activities. *J. Heterocycl. Chem.* **2018**, *55* (4), 791–802. https://doi.org/10.1002/jhet.3112

(15) Liao, L.; Jiang, C.; Chen, J.; Shi, J.; Li, X.; Wang, Y.; Wen, J.; Zhou, S.; Liang, J.; Lao, Y. Synthesis and Biological Evaluation of 1, 2, 4-Triazole Derivatives as Potential Neuroprotectant against Ischemic Brain Injury. *Eur. J. Med. Chem.* **2020**, *190*, 112114. https://doi.org/10.1016/j.ejmech.2020.112114

(16) Janowska, S.; Paneth, A.; Wujec, M. Cytotoxic Properties of 1, 3, 4-Thiadiazole Derivatives—A Review. *Molecules* **2020**, *25* (18), 4309. https://doi.org/10.3390/molecules25184309

(17) Kalidhar, U.; Kaur, A. 1, 3, 4-Thiadiazole Derivatives and Their Biological Activities: A Review. *Res. J.*

Pharm. Biol. Chem **2011**, *4*, 1091–1106.
(18) Ragab, F. A. F.; Abdel-Aziz, S. A.; Kamel, M.; Ouf, A. M. A.; Allam, H. A. Design, Synthesis and Biological Evaluation of Some New 1, 3, 4-Thiadiazine-Thiourea Derivatives as Potential Antitumor Agents against Non-Small Cell Lung Cancer Cells. *Bioorg. Chem.* **2019**, *93*, 103323.
https://doi.org/10.1016/j.bioorg.2019.103323
(19) Aly, A. A.; A. Hassan, A.; Makhlouf, M. M.; Bräse, S. Chemistry and Biological Activities of 1, 2, 4-Triazolethiones—Antiviral and Anti-Infective Drugs. *Molecules* **2020**, *25* (13), 3036.
https://doi.org/10.3390/molecules25133036
(20) Aggarwal, R.; Hooda, M.; Kumar, P.; Torralba, M. C. Visible-Light-Mediated Regioselective Synthesis of Novel Thiazolo [3, 2-b][1, 2, 4] Triazoles: Advantageous Synthetic Application of Aqueous Conditions. *Org. Biomol. Chem.* **2022**, *20* (3), 584–595.
https://doi.org/10.1039/D1OB02194J
(21) Elwahy, A. H.; Shaaban, M. R. Synthesis of Pyrido- and Pyrimido-Fused Heterocycles by Multi-Component Reactions (Part 3). *Curr. Org. Synth.* **2014**, *11* (6), 835–873. https://doi.org/10.2174/15701794110614102311403 9.
https://doi.org/10.2174/1570179411061410231140 39
(22) R Shaaban, M.; HM Elwahy, A. Synthesis of Furo-, Pyrrolo-, and Thieno-Fused Heterocycles by Multi-Component Reactions (Part 1). *Curr. Org. Synth.* **2013**, *10* (3), 425–466.
https://doi.org/10.2174/1570179411310030007
(23) Salem, M. E.; Ahmed, A. A.; Shaaban, M. R.; Shibl, M. F.; Farag, A. M. Regioselective Synthesis and Ab Initio Calculations of Fused Heterocycles Thermally and under Microwave Irradiation. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2015**, *148*, 175–183.
https://doi.org/10.1016/j.saa.2015.03.102
(24) Elwahy, A. H. M.; Ginidi, A. R. S.; Shaaban, M. R.; Farag, A. M.; Salem, M. E. Synthesis of Novel Bis-Thiazoles, Bis-Thienopyridines, and Bis-Triazolothiadiazines Linked to Diphenyl Ether Core as Novel Hybrid Molecules. *Synth. Commun.* **2023**, 1–16.
https://doi.org/10.1080/00397911.2023.2179405
(25) Ginidi, A. R. S.; Shaaban, M. R.; Farag, A. M.; Elwahy, A. H. M. Bis (A-bromo Ketones): Versatile Precursors for Novel Bis (S-triazolo [3, 4-b][1, 3, 4] Thiadiazines) and Bis (Thiazoles). *J. Heterocycl. Chem.* **2015**, *52* (5), 1421–1428.
https://doi.org/10.1002/jhet.2223
(26) Elwahy, A. H. M.; Shaaban, M. R. Synthesis of Heterocycles Catalyzed by Iron Oxide Nanoparticles. *Heterocycles* **2017**, *94* (4), 595–655.
https://doi.org/10.3987/REV-16-854
(27) Elwahy, A. H. M.; Darweesh, A. F.; Shaaban, M. R. Microwave-Assisted Synthesis of Bis (Enaminoketones): Versatile Precursors for Novel Bis (Pyrazoles) via Regioselective 1, 3-Dipolar Cycloaddition with Nitrileimines. *J. Heterocycl. Chem.* **2012**, *49* (5), 1120–1125.
https://doi.org/10.1002/jhet.952
(28) Salem, M. E.; Darweesh, A. F.; Elwahy, A. H. M. Synthesis of Novel Scaffolds Based on Thiazole or Triazolothiadiazine Linked to Benzofuran or Benzo [d] Thiazole Moieties as New Hybrid Molecules. *Synth. Commun.* **2020**, *50* (2), 256–270.
https://doi.org/10.1080/00397911.2019.1694689
(29) Mohamed, M. F.; Mohamed, M. S.; Shouman, S. a; Fathi, M. M.; Abdelhamid, I. A. Synthesis and Biological Evaluation of a Novel Series of Chalcones Incorporated Pyrazole Moiety as Anticancer and Antimicrobial Agents. *Appl. Biochem. Biotechnol.* **2012**, *168* (5), 1153–1162. https://doi.org/10.1007/s12010-012-9848-8.
https://doi.org/10.1007/s12010-012-9848-8
(30) Darweesh, A. F.; Abd El-Fatah, N. A.; Abdelhamid, I. A.; Elwahy, A. H. M.; Salem, M. E. Investigation of the Reactivity of (1 H-Benzo [d] Imidazol-2-Yl) Acetonitrile and (Benzo [d] Thiazol-2-Yl) Acetonitrile as Precursors for Novel Bis (Benzo [4, 5] Imidazo [1, 2-a] Pyridines) and Bis (Benzo [4, 5] Thiazolo [3, 2-a]

Pyridines). *Synth. Commun.* **2020**, *50* (16), 2531–2544.
https://doi.org/10.1080/00397911.2020.1784436
(31) Darweesh, A. F.; El-Fatah, N. A. A.; Abdel-Latif, S. A.; Abdelhamid, I. A.; Elwahy, A. H. M.; Salem, M. E. Synthesis and DFT Studies of Novel Aminoimidazodipyridines Using 2-(3H-Imidazo [4, 5-b] Pyrid-2-Yl) Acetonitrile as an Efficient Key Precursor. *Ark. Online J. Org. Chem.* **2021**, *2021*.
https://doi.org/10.24820/ark.5550190.p011.415
(32) Salem, M. E.; Darweesh, A. F.; Farag, A. M.; Elwahy, A. H. M. 2-Bromo-1-(1H-Pyrazol-4-Yl)Ethanone: Versatile Precursors for Novel Mono-, Bis- and Poly{6-(1H-Pyrazol-4-Yl)-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazines}. *Tetrahedron* **2016**, *72* (5), 712–719.
https://doi.org/10.1016/j.tet.2015.12.024
(33) Elwahy, A. H. M.; Abbas, A. A. Synthesis of N -Pivot Lariat Ethers. *J. Heterocycl. Chem.* **2008**, *45* (1), 1–65. https://doi.org/10.1002/jhet.5570450101.
https://doi.org/10.1002/jhet.5570450101
(34) Di Giacomo, B.; Bedini, A.; Spadoni, G.; Tarzia, G.; Fraschini, F.; Pannacci, M.; Lucini, V. Synthesis and Biological Activity of New Melatonin Dimeric Derivatives. *Bioorg. Med. Chem.* **2007**, *15* (13), 4643–4650.
https://doi.org/10.1016/j.bmc.2007.03.080
(35) Martins, M. A. P.; Cunico, W.; Siqueira, G. M.; Leidens, V. L.; Zanatta, N.; Bonacorso, H. G.; Flores, A. F. C. Regiospecific Synthesis of 1, 2-Bis (Azolyl) Ethanes. *J. Braz. Chem. Soc.* **2005**, *16*, 275–279.
https://doi.org/10.1590/S0103-50532005000200022
(36) Padmavathi, V.; Reddy, B. J. M.; Reddy, B. C. O.; Padmaja, A. Synthesis of a New Class of Keto-Linked Bis Heterocycles. *Tetrahedron* **2005**, *61* (9), 2407–2411.
https://doi.org/10.1016/j.tet.2005.01.018
(37) Wang, C.; Jung, G.-Y.; Batsanov, A. S.; Bryce, M. R.; Petty, M. C. New Electron-Transporting Materials for Light Emitting Diodes: 1, 3, 4-Oxadiazole–Pyridine and 1, 3, 4-Oxadiazole–Pyrimidine Hybrids. *J. Mater. Chem.* **2002**, *12* (2), 173–180.
https://doi.org/10.1039/b106907c
(38) Padmavathi, V.; Reddy, B. J. M.; Subbaiah, D. R. C. V. Bischalcones—Synthons for a New Class of Bis (Heterocycles). *New J. Chem.* **2004**, *28* (12), 1479–1483.
https://doi.org/10.1039/B409968K
(39) Elwahy, A. H. M.; Abbas, A. A. Synthetic Communications : An International Journal for Rapid Communication of Synthetic Organic Chemistry Bis (β -Difunctional) Compounds : Versatile Starting Materials for Novel Bis (Heterocycles). *Synth. Commun.* **2000**, *30* (16), 2903–2921.
https://doi.org/10.1080/00397910008087441
(40) Al-Jumaili, M. H. A.; Hamad, A. A.; Hashem, H. E.; Hussein, A. D.; Muhaidi, M. J.; Ahmed, M. A.; Albanaa, A. H. A.; Siddique, F.; Bakr, E. A. Comprehensive Review on the Bis–Heterocyclic Compounds and Their Anticancer Efficacy. *J. Mol. Struct.* **2023**, *1271*, 133970.
https://doi.org/10.1016/j.molstruc.2022.133970
(41) Mohamed, G. G.; Zayed, E. M.; Hindy, A. M. M. Coordination Behavior of New Bis Schiff Base Ligand Derived from 2-Furan Carboxaldehyde and Propane-1, 3-Diamine. Spectroscopic, Thermal, Anticancer and Antibacterial Activity Studies. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2015**, *145*, 76–84.
https://doi.org/10.1016/j.saa.2015.01.129
(42) Salem, M. E.; Fares, I. M. Z.; Ghozlan, S. A. S.; Abdel-Aziz, M. M.; Abdelhamid, I. A.; Elwahy, A. H. M. Facile Synthesis and Antimicrobial Activity of Bis (Fused 4 H-pyrans) Incorporating Piperazine as Novel Hybrid Molecules: Michael's Addition Approach. *J. Heterocycl. Chem.* **2022**, *59* (11), 1907–1926.
https://doi.org/10.1002/jhet.4525
(43) Wang, C.; Jung, G.-Y.; Hua, Y.; Pearson, C.; Bryce, M. R.; Petty, M. C.; Batsanov, A. S.; Goeta, A. E.; Howard, J. A. K. An Efficient Pyridine-and Oxadiazole-Containing Hole-Blocking Material for Organic Light-Emitting Diodes: Synthesis, Crystal Structure, and Device Performance. *Chem. Mater.* **2001**, *13* (4), 1167–1173.
https://doi.org/10.1021/cm0010250

(44) Jain, M.; Sakhuja, R.; Khanna, P.; Bhagat, S.; Jain, S. C. A Facile Synthesis of Novel Unsymmetrical Bis-Spiro [Indole-Pyrazolinyl-Thiazolidine]-2, 4'-Diones. *Arkivoc* **2008**, *15*, 54–64.
https://doi.org/10.3998/ark.5550190.0009.f07
(45) Antonini, I.; Polucci, P.; Magnano, A.; Sparapani, S.; Martelli, S. Rational Design, Synthesis, and Biological Evaluation of Bis (Pyrimido [5, 6, 1-de] Acridines) and Bis (Pyrazolo [3, 4, 5-Kl] Acridine-5-Carboxamides) as New Anticancer Agents. *J. Med. Chem.* **2004**, *47* (21), 5244–5250.
https://doi.org/10.1021/jm049706k
(46) Radwan, I. T.; Elwahy, A. H. M.; Darweesh, A. F.; Sharaky, M.; Bagato, N.; Khater, H. F.; Salem, M. E. Design, Synthesis, Docking Study, and Anticancer Evaluation of Novel Bis-Thiazole Derivatives Linked to Benzofuran or Benzothiazole Moieties as PI3k Inhibitors and Apoptosis Inducers. *J. Mol. Struct.* **2022**, *1265*, 133454.
https://doi.org/10.1016/j.molstruc.2022.133454
(47) Li, P.; Zhou, J.; Liu, Y.; Wang, X. Design, Synthesis and Bioactivity Evaluation of Novel Thioether Derivatives Containing a Sulfonohydrazide Moiety. *Phosphorus. Sulfur. Silicon Relat. Elem.* **2020**, *195* (12), 976–980.
https://doi.org/10.1080/10426507.2020.1762194
(48) El Ashry, E. S. H.; Kassem, A. A.; Abdel-Hamid, H.; Louis, F. F.; Khattab, S. A. N. And MR Aouad. *Arkivoc* **2006**, *14*, 119–132.
(49) Abbas, A. A.; Dawood, K. M. Chapter Four - Recent Developments in the Chemistry of 1H- and 4H-1,2,4-Triazoles; Scriven, E. F. V, Ramsden, C. A., Eds.; *Advances in Heterocyclic Chemistry*, Academic Press, 2023; Vol. 141, pp 209–273.
https://doi.org/10.1016/bs.aihch.2023.04.002
(50) Riyadh, S. M.; Gomha, S. M. Two Decades of the Synthesis of Mono-and Bis-Aminomercapto [1, 2, 4] Triazoles. *RSC Adv.* **2020**, *10* (42), 24994–25012.
https://doi.org/10.1039/D0RA04208K
(51) Boulechfar, C.; Ferkous, H.; Delimi, A.; Djedouani, A.; Kahlouche, A.; Boublia, A.; Darwish, A. S.; Lemaoui, T.; Verma, R.; Benguerba, Y. Schiff Bases and Their Metal Complexes: A Review on the History, Synthesis, and Applications. *Inorg. Chem. Commun.* **2023**, 110451.
https://doi.org/10.1016/j.inoche.2023.110451
(52) Janowska, S.; Khylyuk, D.; Janowski, M.; Kosikowska, U.; Strzyga-Łach, P.; Struga, M.; Wujec, M. Synthesis and Biological Evaluation of New Schiff Bases Derived from 4-Amino-5-(3-Fluorophenyl)-1, 2, 4-Triazole-3-Thione. *Molecules* **2023**, *28* (6), 2718.
https://doi.org/10.3390/molecules28062718
(53) Shalaby, M. A.; Fahim, A. M.; Rizk, S. A. Microwave-Assisted Synthesis, Antioxidant Activity, Docking Simulation, and DFT Analysis of Different Heterocyclic Compounds. *Sci. Rep.* **2023**, *13* (1), 4999.
https://doi.org/10.1038/s41598-023-31995-w