Design, Synthesis, and Antioxidant Activity Screening of Some New Thiazole and Pyrazolo[5,1-c][1,2,4]triazole Derivatives

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Abstract

Thiazoles and pyrazolo[5,1-c][1,2,4]triazoles have attracted particular attention due to their reported biological and therapeutic effects, so the ultimate objective of the current research is to develop some novel heterocyclic ingredients with thiazole and pyrazolo[5,1-c][1,2,4]triazole constituents that have predicted biological effects. This can be achieved by combining 2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**) with cinnamaldehyde (**2**) to generate two different chemicals **3** or **4**, depending on the catalyst employed. Compound **1** reacts also with furan-2-carbaldehyde (**8**), thiocarbohydrazide (**10**), acetylacetone **15** to afford the respective acrylamide deivative **9**, pyrazolo[5,1-c][1,2,4]triazole-3-thione derivative **13**, and oxopyridine-3-carbonitrile derivative **19**. Also bromination of compound **1** can take place using *N*-bromosuccinimide (NBS) and ammonium acetate to provide 2-bromo-2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**14**). On the other hand, when ethyl cyanoacetate was combined with thiocarbohydrazide (**10**), it resulted in the dihydropyrazolo[5,1-c][1,2,4]triazole-3-thione derivative (**21**), which subsequently reacted with chloroacetic acid **22** and bromo-acetamide derivative **14** to achieve the equivalent pyrazolo[5,1-*c*][1,2,4]triazole derivatives **24** and **26**. Compound **24** can potentially be employed as the precursor for the production of mercaptobut-2-en derivatives **28** and **31**. Applying IR, ^{1}H NMR, and mass spectroscopy, the molecular structure of each constructed product was verified. The antioxidant activity of some of the prepared compounds was assessed using the DPPH free radical scavenging assay in triplicate. Average values were taken into consideration, with ascorbic acid used as the reference standard, and all the investigated substances demonstrated antioxidant activity.

Keywords: Cyanoacetamide, cinnamaldehyde, thiocarbohydrazide, triazole, DPPH, Antioxidant.

1- Introduction

Designing, synthesizing, and creating compounds with the ability to be employed as therapeutics for humans is one of the most important objectives associated with organic and pharmaceutical chemistry. Combinatorial chemistry has enabled access to chemical libraries based on encouraged structures over the past ten years [1], Particular focus is being placed on heterocyclic scaffolds since they are a category of molecules with established medicinal chemistry

applications [2]. There is an abundance of five-membered rings with two heteroatoms, which are biologically active substances. One of these rings is the thiazole ring. Due to its numerous pharmacological purposes, thiazole provides a good pharmacophore nucleus. Antioxidant, analgesic, antibacterial, anticancer, antiallergic, antihypertensive, anti-inflammatory, antimalarial, antifungal, and antipsychotic are a few examples of the biological effects of its derivatives [3,4,5,6,7,8,9]. More than 18 FDA-approved medications contain the thiazole scaffold. Additionally, pyrazolo[5,1-*c*][1,2,4]triazoles are significant heterocyclic ring systems with a variety of biological activities, including antimicrobial [9,10] and anticancer drugs [11]. Likewise, they serve as intermediaries in the production of toners, inks, and other photographic materials such as magenta couplers in photosensitive emulsion layers, which are all photosensitive materials in color [12-15]. Therefore, the main target of this research is to synthesize novel thiazole and pyrazolo[5,1-*c*][1,2,4]triazole derivatives starting from 2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**) and thiocarbohydrazide (**10**) and to examine their biological activity as antioxidants.

2- Experimental

The melting points of each substance were measured using a Gallenkamp melting point instrument. The infrared spectra of potassium bromide discs were recorded using a Pye Unicam SP 3300 and a Shimadzu FT IR 8101 PC infrared spectrophotometer. As an internal reference, tetramethylsilane was used to record the ^{1}H NMR spectra at 300 MHz on a Varian Gemini NMR spectrometer and in deuterated dimethylsulfoxide (DMSO-d6). The results were expressed as δ values. The mass spectra for this study were captured using Shimadzu GCMS-QP 5000 EI and Shimadzu GCMS-QP 1000 EX mass spectrometers at 70 eV. The previously mentioned investigations were carried out by the Regional Centre for Mycology and Biotechnology at Al-Azhar University in Cairo, Egypt, as well as the Microanalytical Centre at Cairo University in Giza, Egypt.

Reagents used: Triethylamine, piperidine, cinnamaldehyde, furfural (furan-2-carbaldehyde), thiocarbohydrazide, acetylacetone, ammonium acetate, ethyl cyanoacetate, chloroacetic acid, and *N*-bromosuccinimide (NBS) were obtained from British Drug Houses (BDH) and EL-Nasr Pharmaceutical and Chemical Co. (ADWIC) in Egypt. Diethyl ether was acquired from Aldrich Chemical Co. The Egyptian company EL-Nasr Pharmaceutical and Chemical Co. (ADWIC) provided the ethanol. In my prior work [16], 2-Cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**), m.p. 110–112°C, was reported. 1,3-Diphenylpropane-1,3-dione (**5**), m.p. 77–78°C, was synthesized in accordance with the published literature [17].

*Reaction between 2-cyano-N-(5-methylthiazol-2-yl)acetamide (**1**) and cinnamaldehyde (**2**)*

Using two distinct basic catalysts, 2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**) and cinnamaldehyde (**2**), interacted to produce two different compounds. When triethylamine (TEA)

was added in small amounts to an ethanolic solution of the cyanoacetamide derivative **1** (0.362 g, 2 mmol) and cinnamaldehyde (**2**) (0.264 g, 2 mmol) and the mixture was then heated under reflux for 5 hours, cooled to an ambient temperature, transferred over an ice-water mixture, and neutralized with dilute hydrochloric acid, The matching pyrrole-3-carbonitrile derivative (**3**) was obtained by filtering off the solid product, drying it, and recrystallizing it from the appropriate solvent. Repeating the above reaction while substituting a few drops of piperidine with trimethylamine resulted in the production of the phenylpenta-2,4-dienamide derivative **4**.

5-Formyl-2,5-dihydro-1-(5-methylthiazol-2-yl)-2-oxo-4-phenyl-1*H*-pyrrole-3-carbonitrile (**3**):

Yellow crystal; yield 86%; m.p. 160ºC; IR (KBr) νmax/cm^{-1}: 2226 (C≡N), 1672, 1605 (2 C=O); ^{1}H NMR (DMSO-d6): δ 2.32 (s, 3H, CH_3), 7.19-7.54 (m, 5H, Ar-H), 7.69 (s, 1H, CH), 7.71 (s, 1H, CH), 8.19 (d, 1H, CHO, *J* =11.4 HZ); MS m/z (%) calculated $C_{16}H_{11}N_3SO_2$: 309.35, found: 309.73 (M^+, 25.31), 281.56 (56.66), 264.10 (86.96), 216.26 (61.85), 156.77 (51.33), 97.97 (100.00).

(2*E*)-2-cyano-*N*-(5-methylthiazol-2-yl)-5-phenylpenta-2,4-dienamide (**4**):

Yellow crystal; yield 92%; m.p. 114-116ºC; IR (KBr) νmax/cm^{-1}: 3431 (NH), 2220 (C≡N), 1685 (C=O); ^{1}H NMR (DMSO-d6): δ 2.22 (s, 3H, CH_3), 6.50 (dd, 1H, CH, *J* = 16.0, 7.3 HZ), 7.03 (s, 1H, CH), 7.22-7.48 (m, 5H, Ar-H and 1H, CH), 9.20 (s, 1H, NH), 9.80 (d, 1H, CH, *J*=7.3 HZ); MS m/z (%) calculated $C_{16}H_{13}N_3SO$: 295.36, found 295.87 (M^+, 16.88), 291.34 (18.83), 247.75 (49.00), 197.98 (55.07), 123.09 (92.61), 120.34 (100.00).

*Synthesis of (1,6-dihydro-1-(5-methylthiazol-2-yl)-2-phenyl-4-styrylpyridin-3-yl)(phenyl)-methanone derivative (**7**)*

A few drops of trimethylamine were added to an ethanolic solution containing the dienamide derivative **4** (0.590 g, 2 mmol) and 1,3-diphenylpropane-1,3-dione (**5**) (0.448 g, 2 mmol). The reaction mixture was refluxed for five hours, then dumped over a solution of ice and water and neutralized with hydrochloric acid. Chemical (**7**) was produced by filtering the generated solid product, rinsing it with water, drying it, and crystallizing it using a compatible solvent.

Yellow crystal; yield 89%; m.p. 80ºC; IR (KBr) νmax/cm^{-1}: 2250 (C≡N), 1681,1600 (2C=O); ^{1}H NMR (DMSO-d6): δ 2.50 (s, 3H, CH_3), 4.88 (s, 1H, CH), 7.23-8.19 (m, 15H, Ar-H and 2H, CH=CH); MS m/z (%) calculated $C_{31}H_{21}N_3SO_2$: 499.59, found 499.26 (M^+, 13.23), 478.31 (72.42), 467.33 (85.01), 396.50 (47.73), 340.97 (100.00), 318.21 (75.67).

*Synthesis of (E)-2-cyano-3-(furan-2-yl)-N-(5-methylthiazol-2-yl)acrylamide (**9**)*

A catalytic amount of piperidine was introduced to a mixture of furan-2-carbaldehyde (**8**) (0.192 g, 2 mmol) and cyanoacetamide derivative **1** (0.362 g, 2 mmol) in ethanol (20 ml). The resultant solution was placed over a mixture of ice and water, followed by neutralization with diluted

hydrochloric acid. To possess acrylamide **9**, the created solid ingredient was extracted, dried, and recrystallized using the adequate solvent.

Brown crystal; yield 62%; m.p. 265ºC; IR (KBr) νmax/cm^{-1}: 3406 (NH), 2218 (C≡N), 1624(C=O); ^{1}H NMR (DMSO-d6): δ 2.22 (s, 3H, CH_3), 5.30 (s, 1H, CH), 6.63, 6.83, 7.00 (m, 3H, furan ring-H), 8.20 (s, 1H, CH), 8.95 (s, 1H, NH); MS m/z (%) calculated $C_{12}H_9N_3SO_2$: 259.29, found: 259.20 (M^+, 25.39), 244.16 (40.58), 214.80 (62.69), 164.93 (100.00), 123.02 (77.46), 79.90 (66.83).

*Reaction between 2-cyano-N-(5-methylthiazol-2-yl)acetamide (**1**) and thiocarbohrdrazide (**10**)*

Thiocarbohydrazide **8** (0.212 g, 2 mmol) was added to a solution of cyanoacetamide derivative **1** (0.362 g, 2 mmol) in ethanol (20 ml). After being refluxed for 5 hours, the reaction mixture was allowed to cool to atmospheric temperature. The created solid was extracted using a filter, rinsed with ethanol, and finally dried. 6-amino-1,2-dihydro-1-(5-methylthiazol-2-yl)pyrazolo[5,1-*c*][1,2,4]triazole-3-thione (**13**), was attained by recrystallization from a suitable chemical solvent.

Brown crystal; yield 83%; m.p. 150ºC; IR (KBr) νmax/cm^{-1}: 3457, 3272 (NH_2), 3205 (NH); ^{1}H NMR (DMSO-d6): δ 2.50 (s, 3H, CH_3), 3.98 (s, 2H, NH_2), 5.25 (s, 1H, CH), 7.14 (s, 1H, CH), 8.67 (s, 1H, NH); MS m/z (%) calculated $C_8H_8N_6S_2$: 252.32, found: 252.58 (M^+, 28.00), 244.12 (20.52), 214.54 (25.05), 149.89 (100.00), 128.75 (63.76), 54.53 (69.79).

*Interaction between 2-cyano-N-(5-methylthiazol-2-yl)acetamide (**1**) and N-bromosuccinimide (NBS)*

Ammonium acetate (0.077 g, 1 mmol) was added to a combination of cyanoacetamide derivative **1** (0.905 g, 5 mmol) and *N*-bromosuccinimide (NBS) (0.890 g, 5 mmol) in 15 ml of dry diethyl ether. After 30 minutes of agitation at 25°C, the resulting combination was filtered and the filtrate was evaporated. After that, the final solid substance was washed with water, dried, and then re-crystallized with the proper liquid solvent to produce 2-bromo-2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**14**).

Brown crystal; yield 74%; m.p. 95ºC; IR (KBr) νmax/cm^{-1}: 3400 (NH), 2268 (C≡N), 1698 (C=O); ^{1}H NMR (DMSO-d6): δ 2.17 (s, 3H, CH_3), 3.31 (s, 1H, CH), 6.56 (s, 1H, CH), 11.03 (s, 1H, NH); MS m/z (%) calculated $C_7H_6N_3SOBr$: 260.11, found: 260.41 (M^+, 22.01), 237.24 (54.44), 207.08 (54.89), 180.22 (100.00), 162.09 (43.82), 107.58 (67.33).

*Reaction of 2-cyano-N-(5-methylthiazol-2-yl)acetamide (**1**) with acetylacetone **15***

Acetylacetone **15** (0.300 g, 3 mmol) and cyanoacetamide derivative **1** (0.362 g, 2 mmol) were heated together at 130°C for 45 minutes before the reaction output was cooled to environmental temperature, cleaned using water, and dried. The 1,2-dihydro-4,6-dimethyl-1-(5-methylthiazol-2-yl)-2-oxopyridine-3-carbonitrile (**19**) was formed by recrystallization from the relevant liquid.

Brown crystal; yield 87%; m.p. 180°C; IR (KBr) νmax/cm^{-1}: 2261 (C≡N), 1694 (C=O); ^{1}H NMR (DMSO-d6): δ 2.08 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 6.57 (s, 1H, CH), 7.15 (s, 1H, CH); MS m/z (%) calculated $C_{12}H_{11}N_3SO$: 245.30, found: 245.24 (M^+, 13.16), 218.91 (44.82), 206.00 (100.00), 179.39 (98.92), 133.14 (57.13), 105.50 (64.32).

*Synthesis of 6-amino-1,2-dihydropyrazolo[5,1-c][1,2,4]triazole-3-thione (**21**)*

Thiocarbohrdrazide (**10**) (1.061g, 10 mmol) was gradually introduced to the 140°C-heated ethyl cyanoacetate (1.697 g, 15 mmol) over a duration of 30 minutes, and the heating process was continued for a further 30 minutes. After this, the resulting mixture proceeded to cool to an ambient temperature. To obtain pyrazolo[5,1-*c*][1,2,4]triazole derivative **21**, the resulting solid that formed was passed through filtration, cleaned with petroleum ether (60/80), dried, and subsequently recrystallized using ethanol.

White crystal; yield 82%; m.p. 140-142°C; IR (KBr) νmax/cm^{-1}: 3482, 3306 (NH_2), 3273, 3206 (2NH); ^{1}H NMR (DMSO-d6): δ 5.65 (s, 2H, NH_2), 7.12 (s, 1H, CH), 8.41 (s, 1H, NH), 8.65 (s, 1H, NH); MS m/z (%) calculated $C_4H_5N_5S$: 155.18, found: 154.94 (M^+, 39.36), 137.91 (55.04), 126.76 (54.53), 116.10 (77.75), 93.76 (60.34), 59.53 (100.00).

*Reaction between 6-amino-1,2-dihydropyrazolo[5,1-c][1,2,4]triazole-3-thione (**21**)and α-halo-carbonyl compounds*

A few droplets of triethylamine were incorporated into an ethanolic solution of the pyrazolo[5,1-*c*][1,2,4]triazole-3-thione derivative **21** (0.310 g, 2 mmol) and the appropriate α-halocarbonyl molecule, either chloroacetic acid **22** (0.189 g, 2 mmol) or 2-bromo-acetamide derivative **14**. The reaction was refluxed for 5 hours before being allowed to cool to ambient temperature, then dumped over an ice-water mixture and neutralized with dilute hydrochloric acid. To obtain the respective pyrazolo[5,1-*c*][1,2,4]triazole derivatives **24** and **26**, the generated solid ingredient was filtered out, dried, and recrystallized from the adequate solvent.

2-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-3-ylthio)acetaldehyde derivative (**24**)

White crystal; yield 96%; m.p. 198°C; IR (KBr) νmax/cm^{-1}: 3457, 3271 (NH_2), 1609 (C=O); ^{1}H NMR (DMSO-d6): δ 3.89 (s, 2H, CH_2), 5.15 (s, 2H, NH_2), 5.26 (s, 1H, CH); MS m/z (%) calculated $C_6H_5N_5SO$: 195.20, found: 195.91 (M^+, 21.02), 181.51 (36.78), 165.71 (100.00), 132.82 (70.82), 110.11 (96.94), 102.92 (57.73).

(2*E*)-2-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-3-ylthio)-3-amino-*N*-(5-methylthiazol-2-yl)acrylamide derivative (**26**)

Yellow crystal; yield 67%; m.p. 136-138°C; IR (KBr) νmax/cm^{-1}: 3306, 3273, 3204, 3176 ($2NH_2$), 2961 (NH), 1641 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.05 (s, 3H, CH_3), 3.32 (s, 2H, NH_2), 4.48 (s, 2H, NH_2), 5.28 (s, 1H, CH), 7.12 (s, 1H, CH), 8.66 (s, 1H, NH).

*Reaction of 2-(6-amino-5H-pyrazolo[5,1-c][1,2,4]triazol-3-ylthio)acetaldehyde derivative (**24**) with active methylene reagents.*

Typical practise:

To a combination of compound **24** (0.390 g, 2 mmol) and a comparable quantity of either ethyl cyanoacetate (0.226 g, 2 mmol) or 1,3-diphenylpropane-1,3-dione (**5**) (0.448 g, 2 mmol) in ethanol (20 ml) , a catalytic amount of triethylamine was introduced. The resultant mixture was subsequently refluxed for 6 hours, then chilled, filtered off, rinsed via ethanol, and dried out. The matching mercaptobut-2-en derivatives **28** and **31**, respectively, were produced by recrystallization using the proper solvent.

(2*E*)-ethyl 3-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-5-yl)-2-(aminomethyl)-4-mercaptobut-2-enoate derivative (**28**)

White crystal; yield 88%; m.p. 230ºC; IR (KBr) νmax/cm^{-1}: 3432, 3327 (NH_2), 1608 (C=O); ^{1}H NMR (DMSO-d6): δ 1.03 (t, 3H, CH_3, *J*= 4.8 Hz), 3.77 (q, 2H, CH_2, *J*= 6.6 Hz), 3.89 (s, 2H, CH_2), 5.26 (s, 2H, NH_2), 8.66 (s, 1H, CH); MS m/z (%) calculated $C_{11}H_{10}N_6SO_2$: 290.30, found: 290.04 (M^+, 24.79), 259.78 (38.22), 232.78 (30.05), 206.72 (43.33), 163.41 (37.93), 107.52 (100.00).

(2*E*)-3-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-5-yl)-2-benzyl-4-mercapto-1-phenylbut-2-en-1-one derivative (**31**)

White crystal; yield 86%; m.p. 185ºC; IR (KBr) νmax/cm^{-1}: 1605 (C=O); ^{1}H NMR (DMSO-d6): δ 3.90 (s, 2H, CH_2), 7.34-8.19 (m, 10H, Ar-H and 1H, CH); MS m/z (%) calculated $C_{21}H_{13}N_5SO$: 383.43, found: 383.36 (M^+, 10.30), 336.25 (63.35), 253.17 (81.02), 221.01 (87.22), 169.84 (89.85), 103.02 (100.00).

3- Results and Discussion

According to the previously described method [16], 2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**) was created by the reaction of 5-methyl-thiazol-2-amine (**I**) and ethyl cyanoacetate, as explained in the following equation (figure 1).

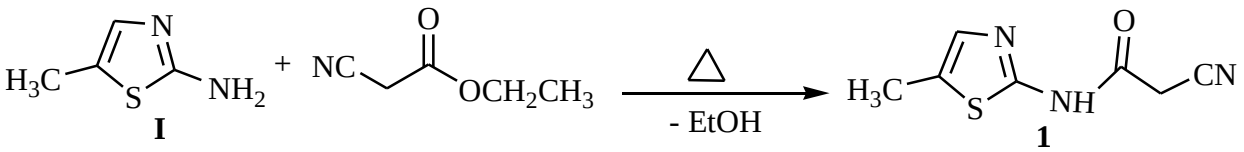
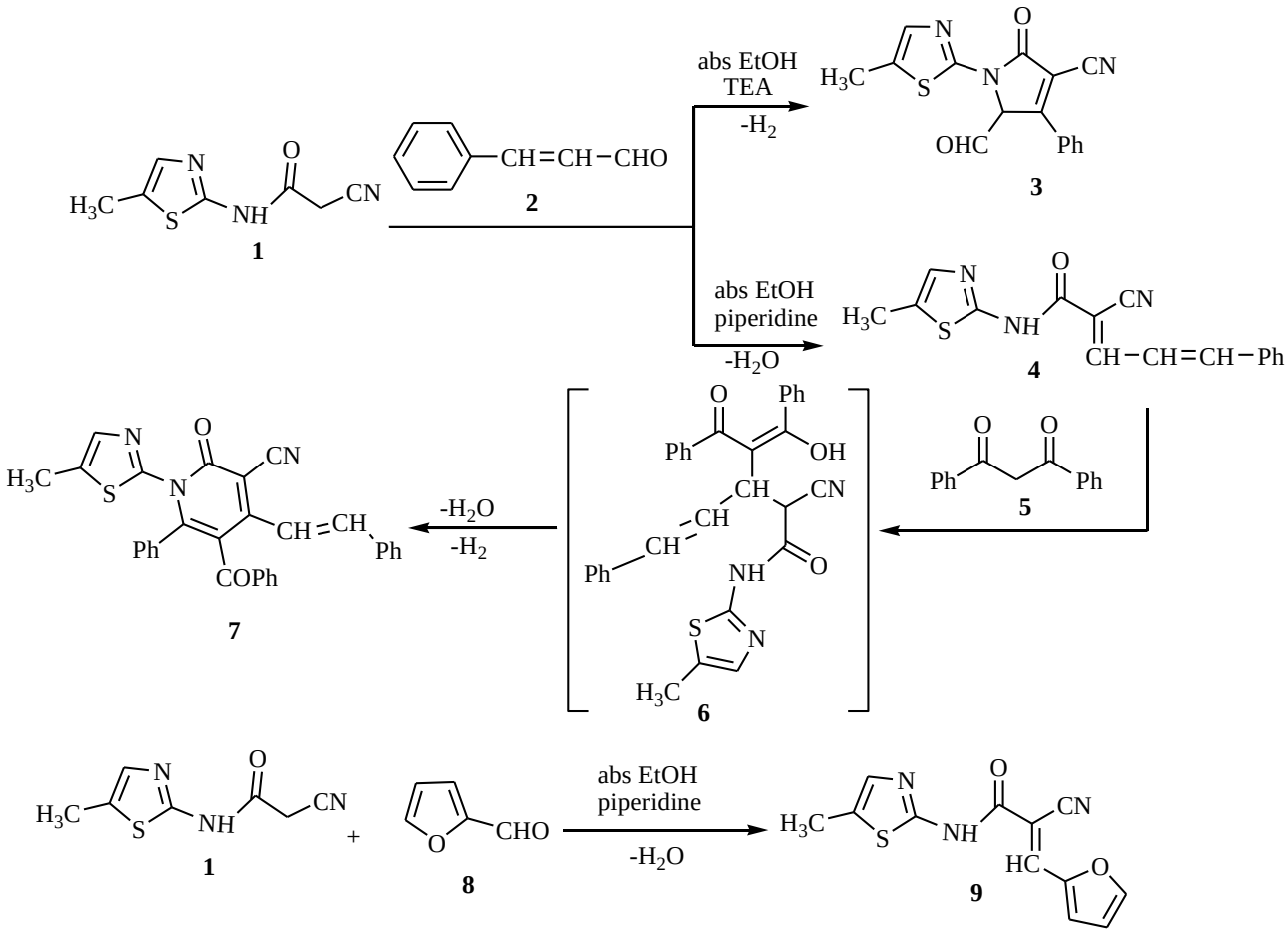


Figure 1

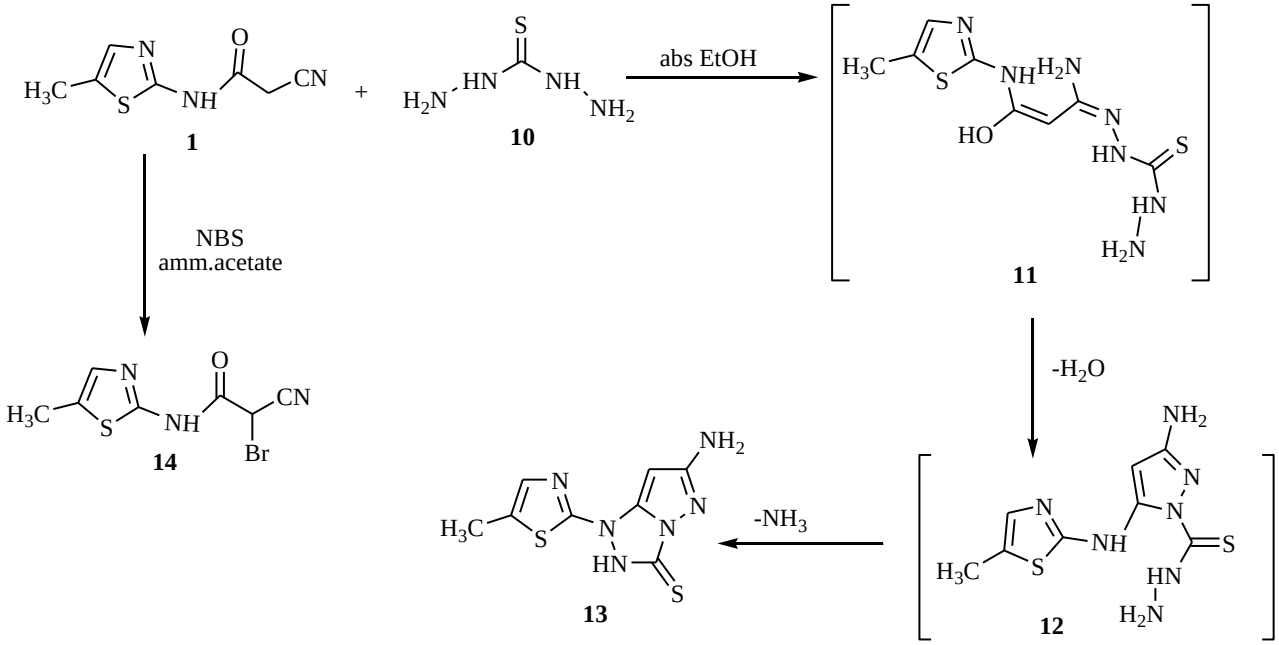


Scheme 1

According to the catalyst applied, the reaction between 2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**1**) and cinnamaldehyde (**2**) takes place in one of two ways. When trimethylamine is used, the reaction takes place via the addition of the amide NH and active methylene groups of compound **1** to the carbon-carbon double bond of compound **2**, resulting in the loss of a hydrogen molecule and the production of the pyrrole-3-carbonitrile derivative **3**. The spectrum data were used to determine the structure of the latter product (see Experimental section). For instance, the IR spectra of chemical compound **3** exhibited the presence of two carbonyl absorption bands at 1672 and 1605 cm^{-1}, as well as one absorption band near 2226 cm^{-1} that is diagnostic of a nitrile function. A peak at m/z (%) 309.73 (25.31) in its mass spectra was identified as the molecular ion. In addition to the anticipated chemical shifts, compound **3**'s ^{1}H NMR spectrum also displayed a doublet signal at δ 8.19 caused by the aldehyde proton. In contrast, when the same reaction was repeated with piperidine added in place of trimethylamine, the water molecule was lost in the process, yielding the phenylpenta-2,4-dienamide derivative **4**. The IR spectra of this derivative showed an NH band at 3431 cm^{-1}, a nitrile absorption band around 2200 cm^{-1}, and a carbonyl band at 1685 cm^{-1}. Compound **4**'s ^{1}H NMR spectrum indicated a signal at δ 9.20 due to an amide NH proton, a doublet of doublets at δ 6.50 due to a CH proton,

as well as an aromatic multiple at δ 7.22–7.48 due to phenyl protons. Additionally, a molecular ion peak at m/z 295.87 (16.88%) was visible in the mass spectrum for this substance.

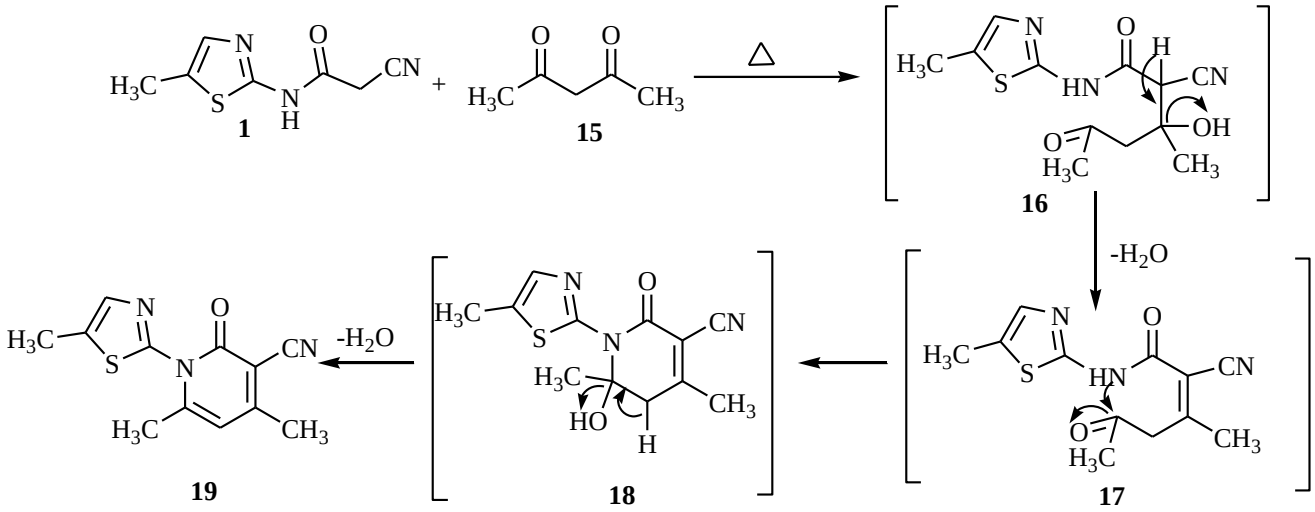
In the presence of catalytic amounts of piperidine and refluxing ethanol, the reaction of compound **4** with 1,3-diphenylpropane-1,3-dione (**5**) produced the condensed product 1,6-dihydro-1-(5-methylthiazol-2-yl)-2-phenyl-4-styrylpyridin-3-yl)(phenyl)methanone derivative (**7**). A nitrile absorption band at 2250 cm^{-1} and two potent carbonyl bands at 1681 and 1660 cm^{-1} were visible in the infrared spectra of compound **7**. As well as an aromatic multiple signal at δ 7.23-8.19 caused by phenyl ring and vinyl protons, were detected in the ^{1}H NMR spectra of compound **7**. Additionally, a molecular ion peak at m/z (%) 499.26 (13.23) was visible in the mass spectrum associated with the substance. Additionally, while refluxing ethanol with a catalytic quantity of piperidine, the cyanoacetamide derivative **1** and furan-2-carbaldehyde (**8**) combine to form the (*E*)-2-cyano-3-(furan-2-yl)-*N*-(5-methylthiazol-2-yl)acrylamide (**9**) (Scheme 1). The IR spectra of the latter product showed two absorption bands near 2218 and 1624 cm^{-1}, respectively, induced by the nitrile and carbonyl groups, as well as an absorption band at 3406 cm^{-1} caused by the NH group. Additionally, a peak in the mass spectrum at m/z 259.20 (25.39) coincided with the molecular ion.



Scheme 2

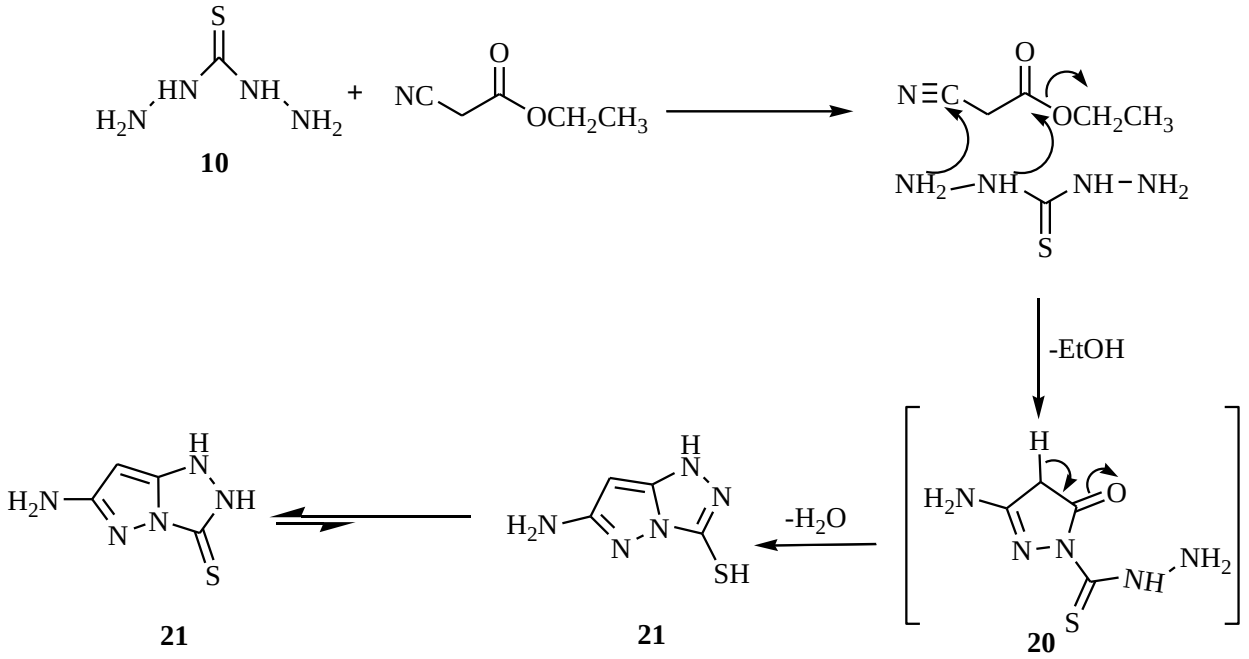
Additionally, this investigation expanded to look into the sensitivity of compound **1** to nitrogen-nucleophilic reagents. Thus, only one isolable product, 6-amino-1,2-dihydro-1-(5-methylthiazol-2-yl)pyrazolo[5,1-*c*][1,2,4]triazole-3-thione (**13**), was produced when compound **1** interacted with an equimolar quantity of thiocarbohydrazide (**10**) in refluxing ethanol (Scheme 2). The thiocarbohydrazide (**10**) was simply added to the CN group in compound **1** to produce the intermediate **11**, which was then followed by the elimination of water to produce the

intermediate **12**. This was then converted into compound **13** by the loss of ammonia. Compound **13**'s IR spectra showed two absorption bands at 3457 and 3272 cm^{-1}, which are typical of the NH_2 group, and one absorption band at 3205 cm^{-1}, which is typical of the NH function. Two D_2O-exchangeable signals for NH_2 and NH protons were visible in compound **13**'s ^1H NMR spectrum at δ 3.98 and 8.67, respectively. Additionally, a molecular ion peak was visible in the mass spectrum of the substance at m/z (%) 252.58 (28.00). The reaction between *N*-bromosuccinimide (NBS) and the cyanoacetamide derivative **1**, in the presence of ammonium acetate, produces 2-bromo-2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**14**) as the end product. The IR spectrum of the isolated product **14** indicates the presence of three bands of absorption near 3400, 2268, and 1698 cm^{-1} that are diagnostic for the NH, nitrile, and carbonyl functions, respectively. Additionally, according to its ^1H NMR spectra, a singlet signal at δ 3.31 due to a CH-Br proton is displayed as well as its mass spectrum demonstrated a peak for a molecular ion at m/z 260.41 (22.01%), Scheme 2.



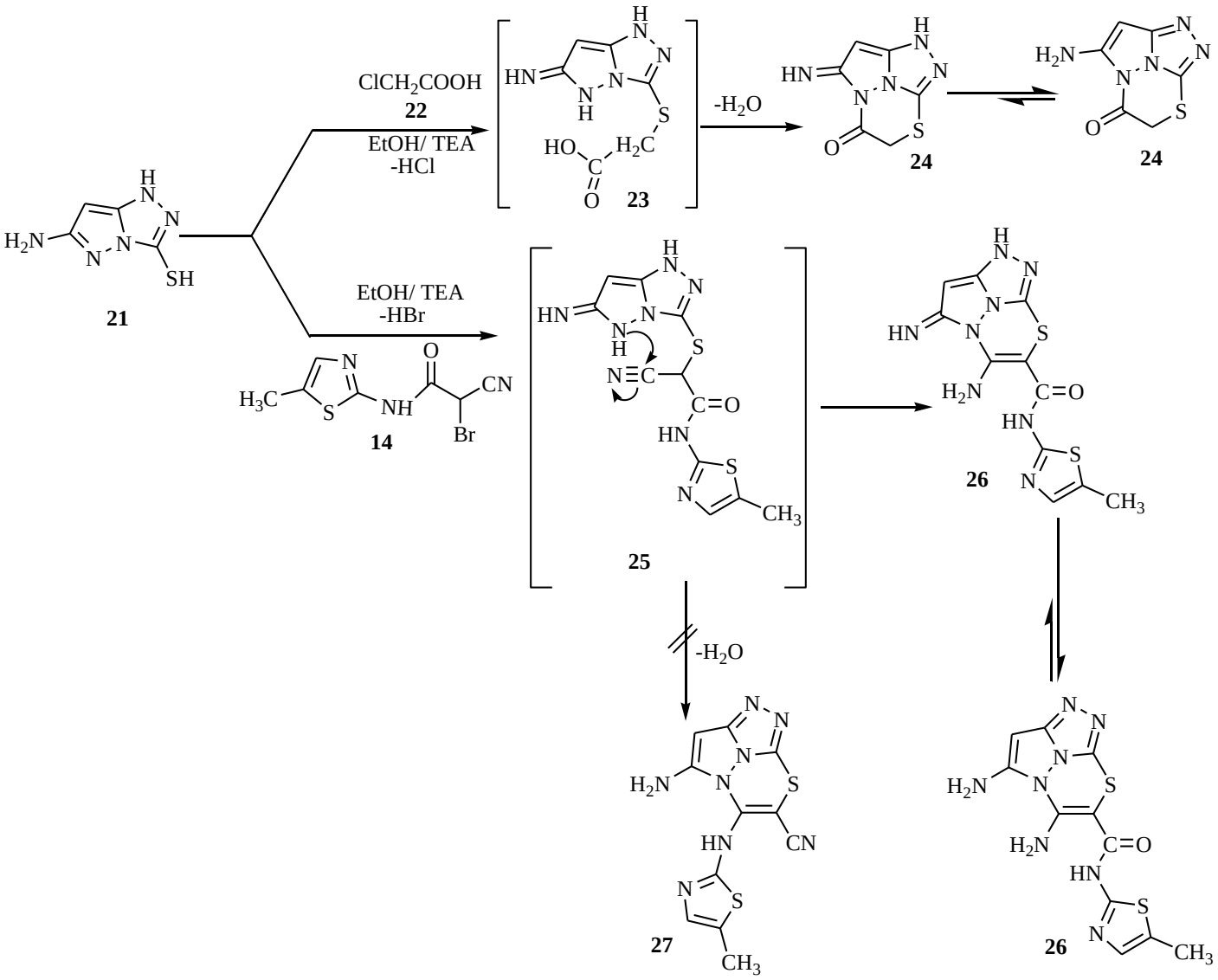
Scheme 3

Acetylacetone **15** was used to treat component **1**, producing the equivalent pyridine-3-carbonitrile derivative **19.** This product is anticipated to form via intermediate **16**, followed by water elimination to produce intermediate **17**, which undergoes facile intramolecular cyclization with another water molecule elimination to generate compound **19** (Scheme 3). Based on its spectral data, the structure of the separated product was determined. For instance, the IR spectra of the resulting isolated compound **19** demonstrated the presence of a carbonyl absorption band at 1694 cm^{-1} and an absorption band near 2261 cm^{-1} that is typical of a nitrile function. Compound **19**'s ^1H NMR spectrum displayed three singlet signals for the three methyl groups at δ 2.08, 2.34, and 2.49, and a singlet signal for the pyridine ring proton at C-3 at δ 7.15. Additionally, the peak in its mass spectrum at m/z 245.24 (13.16%), which matched its molecular ion, was visible.



Scheme 4

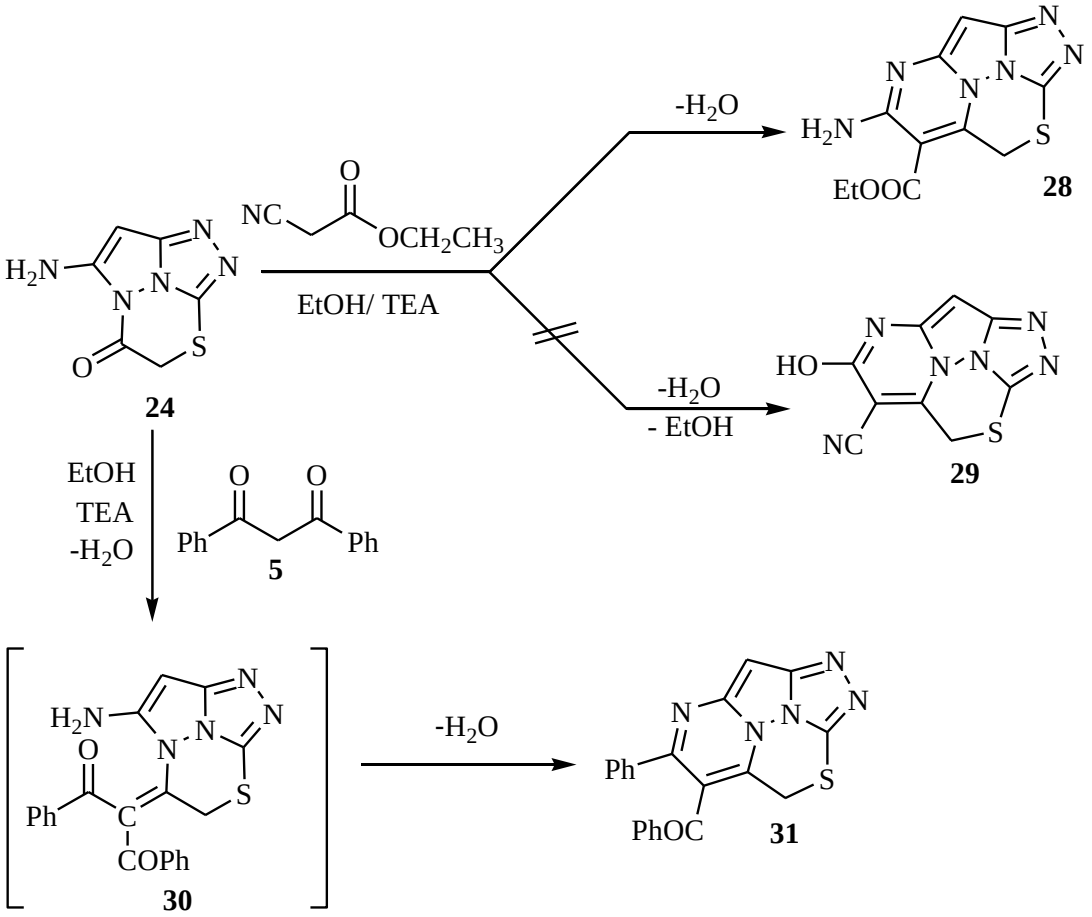
Additionally, this effort included the synthesis of other pyrazolo[5,1-*c*][1,2,4]triazole derivatives. As illustrated in Scheme 4,6-amino-1,2-dihydropyrazolo[5,1-*c*][1,2,4]triazole-3-thione (**21**) was made by combining ethyl cyanoacetate with thiocarbohydrazide (**10**) at the latter's melting point of 140–150°C. The reaction was carried out by the straightforward addition of thiocarbohydrazide (**10**) to the nitrile and carbonyl groups in ethyl cyanoacetate with the elimination of ethanol to produce the intermediate **20**, which then followed by water elimination to give the compound **21**. Compound **21**'s IR spectra provided two bands at 3482 and 3306 cm^{-1}, which are characteristic of NH_2 group, and two bands at 3273 and 3206 cm^{-1}, which are characteristic of two NH functions. Compound **21**'s ^1H NMR spectrum exhibited three D_2O-exchangeable signals at δ 5.65, 8.41, and 8.65 for NH_2 and two NH protons, respectively, and a singlet signal at δ 7.12 for the pyrazole ring proton. Additionally, the molecular ion peak in its mass spectrum was found at m/z (%) 154.94 (39.36).



Scheme 5

The current research proceeded further to examine the susceptibility of compound **21** to α-halocarbonyl compounds. Thus, with the objective of producing only one isolable product, 2-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-3-ylthio)acetaldehyde derivative (**24**), compound **21** interacted with an equimolar amount of chloroacetic acid **22** in refluxing ethanol with a catalytic quantity of triethylamine. Comparable to this, when compound **21** was combined with the 2-bromo-acetamide derivative **14**, the result was a single product (as determined by TLC), for which the two structures **26** and **27** as described in Scheme 5 appeared to be feasible. However, the spectral information of the reaction result precluded the other alternative structure **27** and was only compatible with (2*E*)-2-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-3-ylthio)-3-amino-*N*-(5-methylthiazol-2-yl)acrylamide derivative (**26**), its IR spectrum indicated two amino groups with four absorption bands at 3306, 3273, 3204, and 3176 cm^{-1}, an amide-NH group with one absorption band at 2961 cm^{-1}, and a carbonyl group with one absorption band at 1641 cm^{-1}.

Furthermore, its ^{1}H NMR spectrum showed three D_2O-exchangeable signals representing two NH_2 and the NH protons, respectively, at δ 3.32, 4.48, and 8.66.



Scheme 6

In an ethanolic triethylamine solution that was being refluxed, compound **24** interacted with ethyl cyanoacetate to produce (2*E*)-ethyl 3-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-5-yl)-2-(aminomethyl)-4-mercaptobut-2-enoate derivative (**28**) as depicted in Scheme 6. In addition to one carbonyl absorption band at 1608 cm^{-1}, the IR spectrum of compound **28** showed two bands of absorption caused by amino groups at 3432 and 3327 cm^{-1}. Compound **28**'s ^{1}H NMR spectrum revealed a triplet signal at δ 1.03, a quartet at δ 3.77 that were indicative of the ethyl ester group, and a D_2O-exchangeable signal at δ 5.26 that was diagnostic for the NH_2 group. A molecular ion peak was observed in compound **28**'s mass report at m/z 290.04 (24.79%). The proposed structure **28** is supported by the spectral analysis results, which rule out the other probable structure **29**. Under the same reaction conditions, compound **24** interacts with 1,3-diphenylpropane-1,3-dione (**5**) to produce (2*E*)-3-(6-amino-5*H*-pyrazolo[5,1-*c*][1,2,4]triazol-5-yl)-2-benzyl-4-mercapto-1-phenylbut-2-en-1-one (**31**) via the intermediate **30** (Scheme 6).

4- Evaluation of Antioxidant Activity using DPPH scavenging

Antioxidant Assay:

At the Regional Centre for Mycology and Biotechnology (RCMB) at Al-Azhar University, the antioxidant activity of the substance was evaluated using the DPPH free radical scavenging test in triplicate, and the average data was taken into consideration.

DPPH Radical Scavenging Activity:

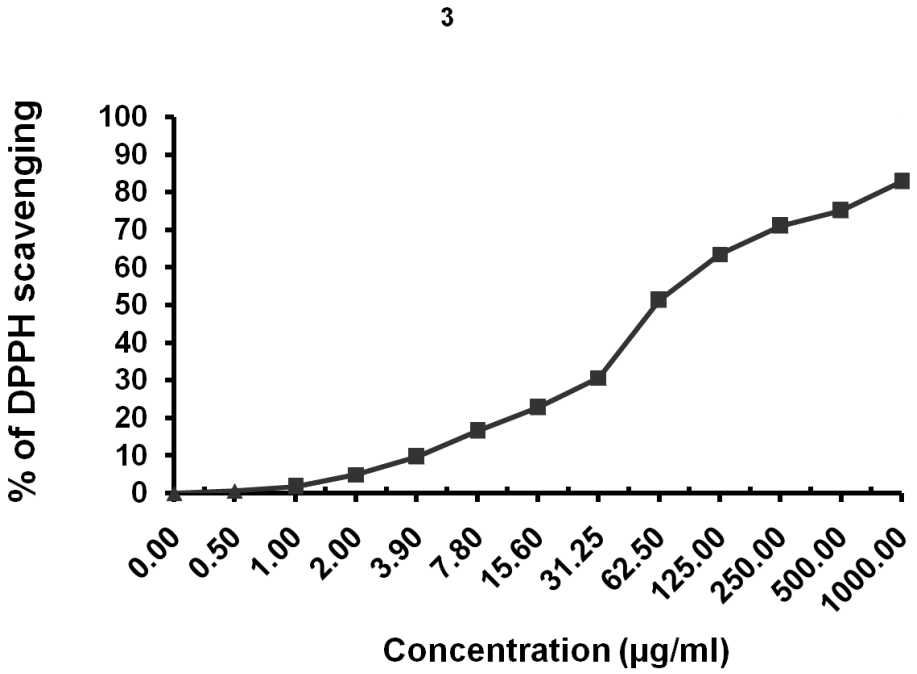
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was created as a freshly prepared 0.004% (w/v) methanol solution and kept at 10°C in the dark. The test substance was made into a methanol solution. 3 mL of the DPPH solution and a 40 µL aliquot of the methanol solution were combined. Using a UV-visible spectrophotometer (Milton Roy Spectronic 1201), absorbance measurements were taken instantly. Once the absorbance had settled (after 16 minutes), the decline in absorbance at 515 nm was monitored constantly, with data being captured at 1-minute intervals. Ascorbic acid was used as a reference chemical, and both its absorbance and that of the DPPH radical without an antioxidant (control) were also assessed. Three replicates of each determination were made, and the average was calculated [18-20]. The DPPH radical's percentage inhibition (PI) was then computed according to the formula:

$$PI = [\{(AC- AT)/ AC\} \times 100] \quad (1)$$

Where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample+DPPH at t = 16 min.

The 50% inhibitory concentration (IC50), the concentration required to inhibit DPPH radical by 50%, was estimated from graphic plots of the dose response curve**.**

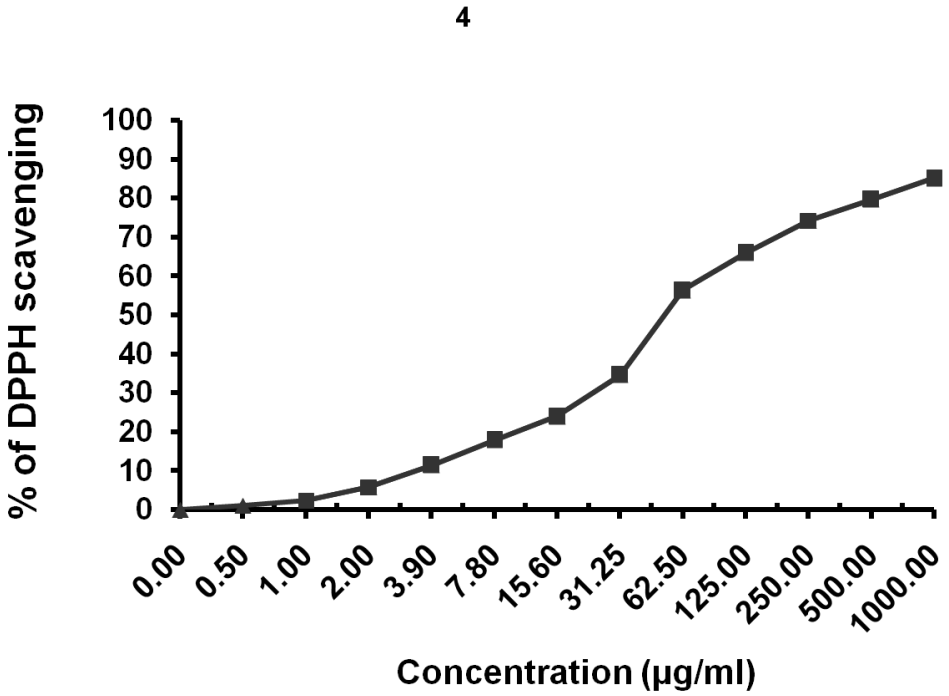
Compound (**3**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**82.86**	**0.62**
500	**75.13**	**0.51**
250	**71.08**	**0.84**
125	**63.45**	**1.23**
62.5	**51.29**	**1.47**
31.25	**30.67**	**1.85**
15.6	**22.84**	**1.02**
7.8	**16.53**	**0.69**
3.9	**9.72**	**0.34**
2	**4.89**	**0.27**
1	**1.78**	**0.36**
0.5	**0.65**	**0.21**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 60.54± 3.17 µg/ml.

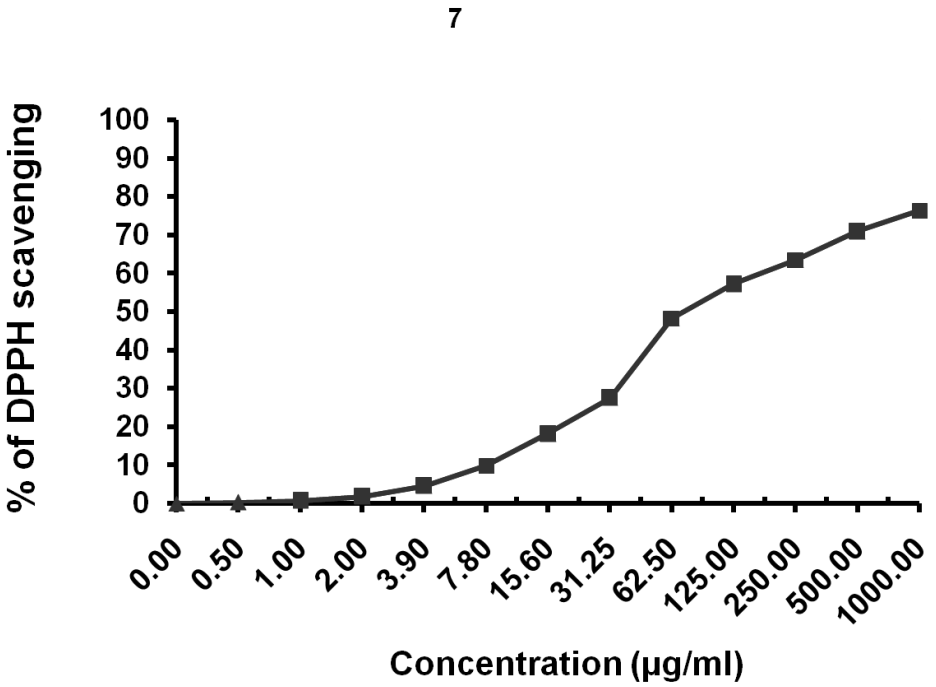
Compound: (**4**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**85.03**	**0.31**
500	**79.58**	**0.54**
250	**74.16**	**0.37**
125	**65.92**	**1.06**
62.5	**56.34**	**1.92**
31.25	**34.58**	**1.86**
15.6	**23.95**	**0.63**
7.8	**17.86**	**0.42**
3.9	**11.48**	**0.06**
2	**5.77**	**0.19**
1	**2.34**	**0.28**
0.5	**1.08**	**0.16**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 =53.39 ± 2.95 µg/ml.

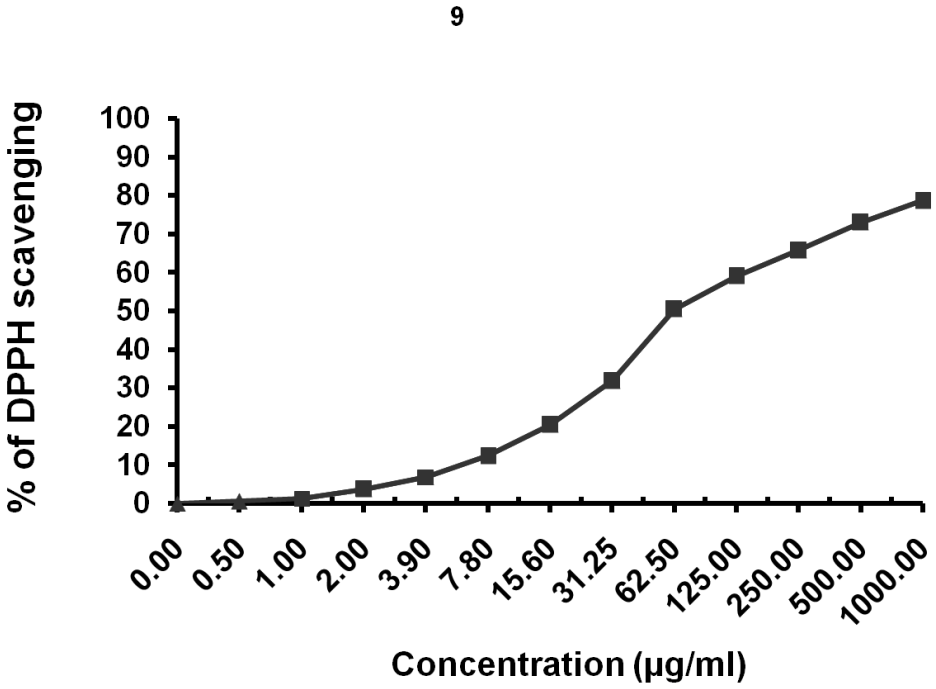
Compound: (**7**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**76.29**	**0.57**
500	**70.88**	**0.68**
250	**63.45**	**0.28**
125	**57.19**	**1.75**
62.5	**48.20**	**1.46**
31.25	**27.46**	**0.82**
15.6	**18.14**	**1.98**
7.8	**9.87**	**0.41**
3.9	**4.53**	**0.65**
2	**1.79**	**0.17**
1	**0.68**	**0.16**
0.5	**0.27**	**0.05**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 75.01 ± 4.57 µg/ml.

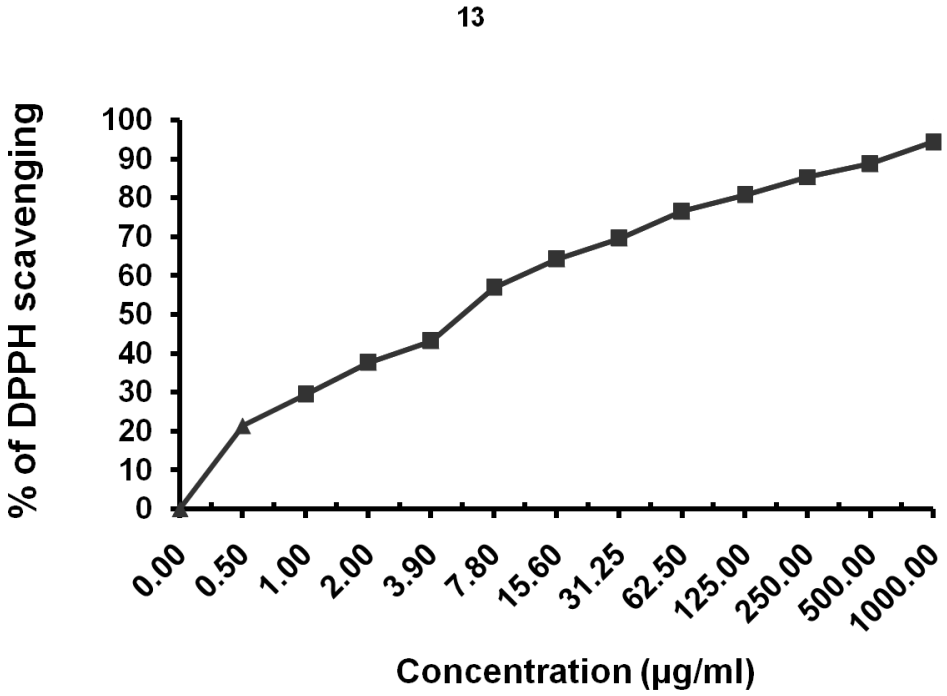
Compound: (**9**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**78.61**	**0.59**
500	**72.95**	**0.73**
250	**65.74**	**0.32**
125	**59.02**	**0.64**
62.5	**50.43**	**1.09**
31.25	**31.85**	**1.91**
15.6	**20.42**	**0.84**
7.8	**12.39**	**0.73**
3.9	**6.84**	**0.08**
2	**3.75**	**0.29**
1	**1.29**	**0.35**
0.5	**0.58**	**0.14**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 61.78 ± 3.42 µg/ml.

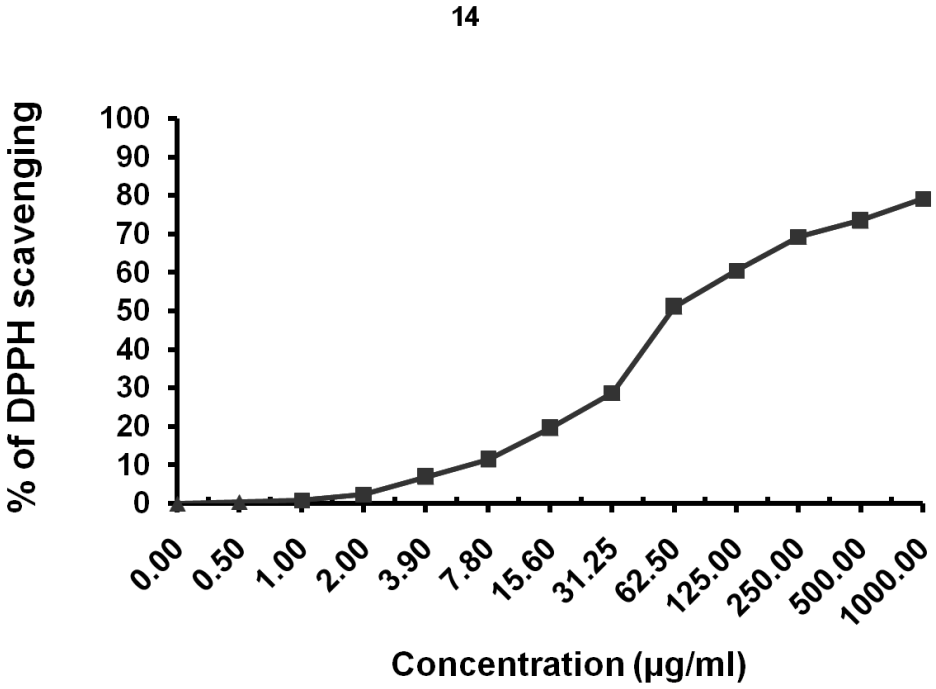
Compound: (**13**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**94.26**	**0.88**
500	**88.67**	**0.71**
250	**85.29**	**0.53**
125	**80.63**	**0.49**
62.5	**76.46**	**0.22**
31.25	**69.52**	**0.41**
15.6	**64.17**	**0.79**
7.8	**56.89**	**0.43**
3.9	**43.18**	**0.67**
2	**37.56**	**0.45**
1	**29.40**	**0.72**
0.5	**21.35**	**0.49**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 5.84 ± 0.41 µg/ml.

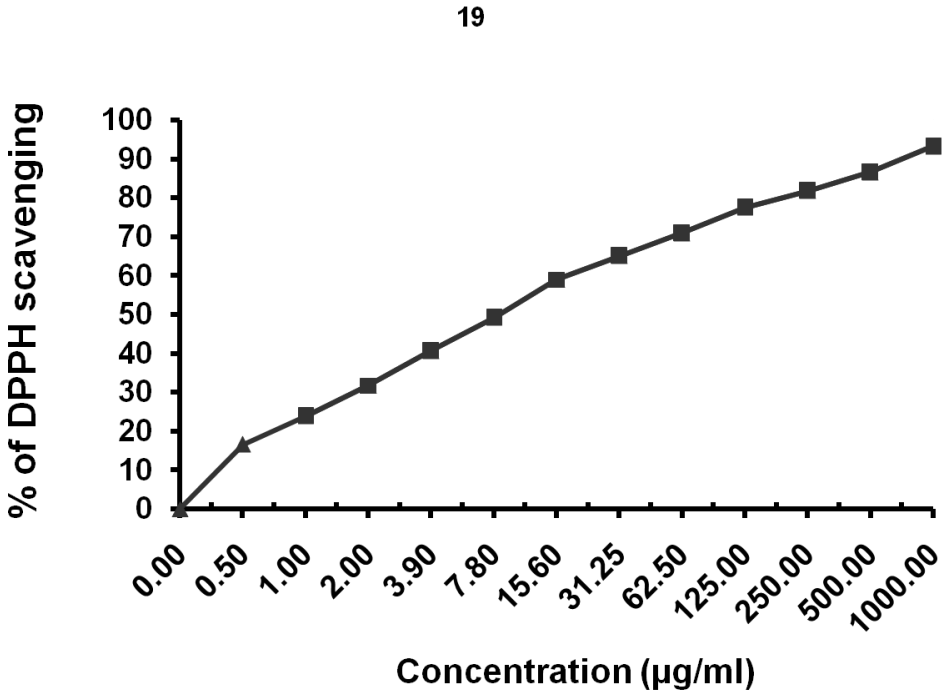
Compound: (**14**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**79.08**	**0.46**
500	**73.46**	**0.28**
250	**69.23**	**0.45**
125	**60.45**	**0.93**
62.5	**51.07**	**1.21**
31.25	**28.72**	**0.94**
15.6	**19.56**	**0.28**
7.8	**11.43**	**0.65**
3.9	**6.95**	**0.29**
2	**2.34**	**0.18**
1	**0.85**	**0.32**
0.5	**0.43**	**0.11**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 61.00 ± 3.29 µg/ml.

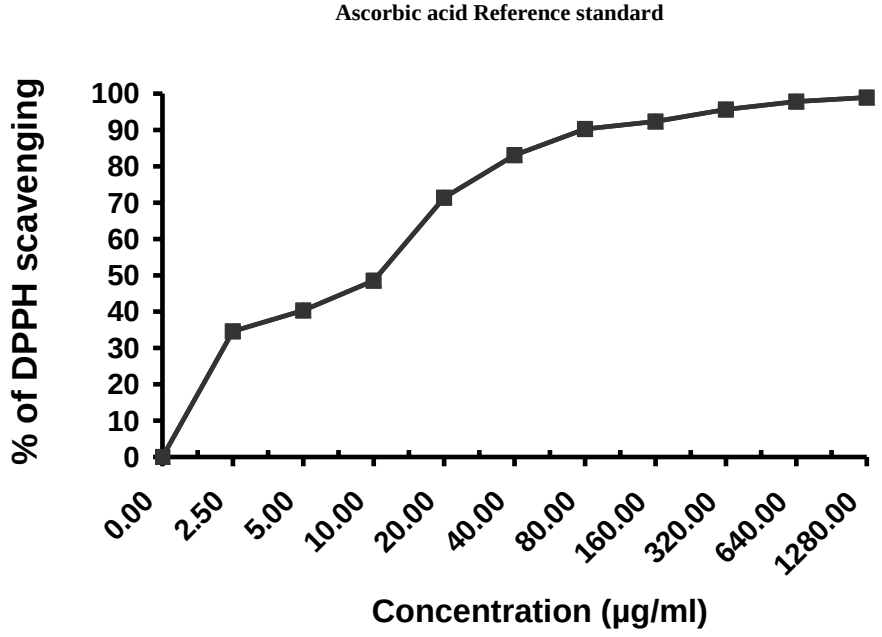
Compound: (**19**)



Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	**93.17**	**0.51**
500	**86.53**	**0.72**
250	**81.76**	**0.48**
125	**77.42**	**0.16**
62.5	**70.91**	**0.87**
31.25	**65.08**	**0.64**
15.6	**58.94**	**0.86**
7.8	**49.18**	**0.76**
3.9	**40.67**	**0.59**
2	**31.72**	**0.43**
1	**23.86**	**0.29**
0.5	**16.54**	**0.18**
0	**0**	**0**

The sample showed an antioxidant activity under these experimental conditions with IC50 = 8.46 ± 0.78 µg/ml.

Compound: Ascorbic acid Reference standard



Sample conc. (µg)	DPPH scavenging %	S.D.
1280	98.91	0.74
640	97.83	0.39
320	95.64	1.22
160	92.31	0.87
80	90.25	0.41
40	83.09	1.95
20	71.38	1.39
10	48.52	2.64
5	40.36	0.82
2.5	34.57	0.79
0	0	

The sample showed an antioxidant activity under these experimental conditions with IC50 = 10.61 ± 0.75 µg/ml.

5- Conclusions

The results of the current investigation point to the following conclusion.

Some novel heterocyclic ingredients with thiazole and pyrazolo[5,1-c][1,2,4]triazole moieties have been produced, such as pyrrole-3-carbonitrile, phenylpenta-2,4-dienamide, acrylamide, pyrazolo[5,1-*c*][1,2,4]triazole-3-thione, oxopyridine-3-carbonitrile derivatives. Moreover, facile bromination of chemical **1** by NBS yielding 2-bromo-2-cyano-*N*-(5-methylthiazol-2-yl)acetamide (**14**). Ethyl cyanoacetate, on the other hand, when mixed with thiocarbohydrazide (**10**), produced the dihydropyrazolo[5,1-*c*][1,2,4]triazole-3-thione derivative (**21**), which was then used to produce pyrazolo[5,1-*c*][1,2,4]triazole derivatives and mercaptobut-2-en derivatives. Modern spectroscopic methods were used to explain the structures of the developed chemicals. The majority of the synthetic ingredients employed in the present investigation demonstrated antioxidant activity when tested in triplicate using the DPPH free radical scavenging assay, with ascorbic acid as the reference standard.

6- Acknowledgments

The Regional Center for Mycology and Biotechnology at Al-Azhar University and the Micro-analytical Center of Cairo University are acknowledged by the author.

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