

Synthesis, Characterization, Antimicrobial, and Antioxidant Activity of Novel Heterocyclic Compounds Containing a Pyrazolone Core Moiety

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Abstract

Background: Polycyclic heterocyclic compounds are highly relevant in medicinal chemistry because of their structural diversity and broad spectrum of biological activity. One of these is pyrazolone-based systems, which have become multifunctional scaffolds with considerable pharmacological potential. **Objective:** The objective of the current project was to develop, prepare, and test a set of new heterocyclic compounds containing a pyrazolone framework, with a focus on targeting antimicrobial and antioxidant activities. **Methods:** Pyrazole carboxaldehyde precursor was previously obtained from ascorbic acid. The precursor was reacted through numerous condensation, cyclization, and multi-component reactions to obtain structurally diverse heterocyclic derivatives. Elemental analysis, infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy were all used to completely characterize the synthesized compounds. The agar well-diffusion technique was used to determine the antimicrobial activity against standard Gram-positive and Gram-negative bacterial strains, and the fungal strain of *Candida albicans*. Compare-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay was used to assess the antioxidant activity, and the IC₅₀ was determined to compare the relative antioxidant activity. **Results:** The biological screening indicated that the synthesized compounds were selectively antifungal against *Candida albicans*, with minimal or no antibacterial activity. Several derivatives showed moderate to strong antioxidant activity depending on concentration. The structure-activity relationship analysis showed that heterocyclic fusion motifs and the inclusion of electron-donating substituents significantly influenced the modulation

of biological activity. **Conclusion:** The results demonstrate that the pyrazolone scaffold is an attractive platform on which novel heterocyclic compounds with potential as antifungal and antioxidant agents can be developed.

Keywords

Pyrazolone, Heterocyclic, Antimicrobial, Antioxidant, Synthesis, Biological Testing

1. Introduction

1.1. Scientific Background

Different heterocyclic compounds containing nitrogen, sulfur, or oxygen are biologically significant and of therapeutic interest due to their potential physical and chemical properties [1] [2]. Oxygen- and nitrogen-containing heterocyclic compounds have gained considerable importance due to their applications in multiple fields [1]-[3].

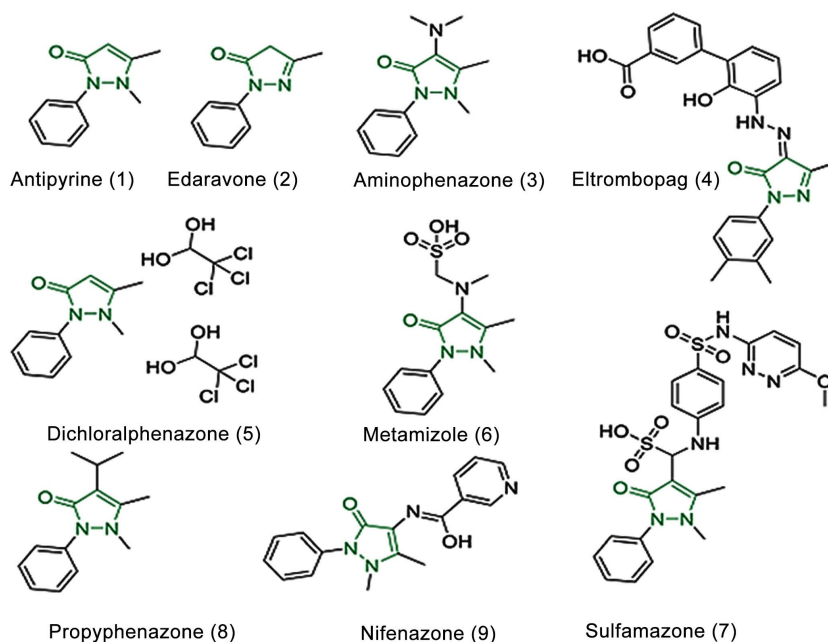


Figure 1. Several medications with a pyrazolone core.

The pyrimidine compounds, as components of nucleic acids, are significant in pharmaceutical chemistry, as they have various applications, including bactericidal [4], fungicidal [5], analgesic [6], anti-inflammatory [7], and antitumor agents [8]. At the same time, the pyrazole nucleus exhibits diverse pharmacological applications **Figure 1**: antimicrobial agents [9] [10], anticancer agents [11], and anti-inflammatory agents [12] [13]. Research in recent years has shown that the pyrazolone core is a versatile scaffold [14] to develop substances with various types of

biological action, including antimicrobial [15], antitubercular [16] [17], antiviral [18], anticancer [18] [19], analgesic [20], anti-inflammatory [21], antioxidant [22], and anti-diabetic [23]. This broad spectrum of bioactivity renders pyrazolone a versatile scaffold that can interact with a variety of biological targets via coordination with metal ions, hydrogen bonding, and π - π interactions [24] [25].

These activities of the pyrazolone moiety prompted us to synthesize corresponding pyrazole derivatives with structural modifications [26] [27]. In continuation of our research on the synthesis of heterocyclic biologically active molecules [23], we have designed and synthesized a series of novel heterocycles using 5-oxo-1-phenyl-4-(2-phenylhydrazone)-4,5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde, intending to develop antimicrobial and antioxidant agents. The development of such chemicals is especially important, since new chemotypes with better pharmacological profiles are required due to increasing antimicrobial resistance and oxidative stress-related illnesses [28]-[30].

1.2. Significance of Heterocyclic Scaffolds in Medicinal Chemistry

Another of the most prevalent structural classes in the field of modern medicinal chemistry is heterocyclic structures [31] [32], which comprise a significant proportion of drugs approved clinically. They are notable because of the capacity of heteroatoms like nitrogen, oxygen, and sulfur to control the electronic distribution, polarity, and hydrogen-bonding potential, which consequently dictate molecular recognition in biological targets [33] [34]. The addition of heterocycles usually enhances the pharmacokinetic properties, such as solubility, metabolic stability, and membrane permeability, thus leading to an improvement in drug-like properties [35].

One of the classes of heterocycles that has remained of interest because of its structural rigidity and synthetic flexibility is the five-membered heterocyclic ring systems, including pyrazole and pyrazolone [5] [36]-[43].

Antimicrobial and antioxidant compounds could offer synergistic therapeutic potential; therefore, by acting on the pathogen, they simultaneously prevent proliferation and reduce host cellular damage [44].

As a drug-discovery tool, bivalent biologic scaffolds are highly relevant since they improve the combination therapy requirement and decrease the tendency of drugs to interact with each other [45]. Such applications are especially well-adapted to pyrazolone derivatives that are redox-active and have demonstrated the ability to be compatible with various heterocyclic fusions. As a result, the systematic design of pyrazolone-based hybrid molecules is still a promising strategy for developing next-generation therapeutic agents [46] [47].

1.3. Rationale

Structural alteration of the pyrazolone core can enhance potency and selectivity [48]. In particular, the addition of heteroatoms and fused ring systems may affect lipophilicity and bioavailability, enhance binding affinity to biological targets, and

modify electron distribution [49] [50]. Thus, a rational strategy to identify compounds with both antibacterial and antioxidant properties is the strategic derivatization of pyrazolone derivatives.

1.4. Knowledge Gap

Limited research combines synthesis, antimicrobial screening, and antioxidant profiling within a single coherent SAR. Despite the numerous reported pyrazolone derivatives, few studies linking chemical structure with either biological result have been reported.

Besides, few reports examine antifungal selectivity with radical-scavenging performance, even though they have therapeutic implications.

1.5. Aim

We had the objective of synthesizing new pyrazolone-based heterocycles and determining their antimicrobial and antioxidant effects, with the hypothesis that the heterocyclic fusion increases the activity.

2. Methodology

2.1. Materials, Reagents, and Instruments

Melting points were determined using a Tottoli (Büchi) apparatus and were not corrected. IR (KBr) spectra were recorded on a Perkin-Elmer 580 VB spectrophotometer, and ^1H -NMR spectra (CDCl_3) and (DMSO-d_6) were obtained on a Camica 250 Hz spectrometer using TMS as an internal standard. Microanalyses were performed in microanalytical units in the Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt. The antimicrobial activities were determined at the Pharmaceutical Science Park Unit (PSPU), Faculty of Pharmacy, Alexandria University. Antioxidant activities of the tested compounds were determined at Nawah Scientific Center, Al Mokattam, Cairo, Egypt. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel 60 f 254 plates. Unless otherwise noted, all reagents and solvents were of analytical grade and were used without additional purification. Glassware was oven-dried before use to exclude moisture, and all experiments were conducted under controlled and reproducible conditions.

2.2. Synthetic Procedures

The initial compound, 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl-3-carbaldehyde (1), was synthesized in a prior procedure. Later derivatives (2-16) were prepared as per the protocols in Section 3 of the original document, and these derivatives were synthesized by condensation, cyclization, and multicomponent reactions of thiosemicarbazone, thioglycolic acid, hydrazine, hydroxylamine, ethyl cyanoacetate, thiourea, acetylacetone, etc.

All reactions were optimized for solvent, temperature, and reaction time in or-

der to optimize yield and purity. Purification was performed through recrystallization or column chromatography, and structural elucidation was achieved by spectroscopy.

2.3. Chemistry

5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde (1) was prepared according to the literature procedure, m.p. 140 - 141; Lit. m.p. 140 - 141 [26] [27]

(2E)-2-((5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl) methylene)hydrazine carbothioamide (2). A solution of compound **1** (1 g) in ethanol (30 ml) was treated with thiosemicarbazide (1 g) and a few drops of acetic acid. The reaction mixture was boiled under reflux for 2 hours, concentrated, and cooled (yield 0.5 g). It was recrystallized from ethanol in yellow needles, m.p. 240 °C - 241 °C; IR (KBr), $\nu_{\max}$ = 3116 (NH), 1732 (lactone C=O), 1680 (OCN), 1352 cm^{-1} (CS); $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.62 (1H, s, NH), 8.12 (1H, s, C₅-H), 6.76 (2H, s, NH₂), 7.22 - 7.98 (10H, m, Ar H). Elemental Analysis (%) Calcd for C₁₇H₁₅N₇OS (365): C, 55.88; H, 4.14; N, 26.83; Found: C, 55.22; H, 4.77; N, 26.46.

5-oxo-2-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-1,3,4-thiadiazinane-4-carbothioamide (3). A mixture of **(2)**, (2.6 g, 5 mmol) and 2-mercaptoacetic acid (0.46 g, 5 mmol) was stirred in dry benzene (25 mL) for 15 min, then refluxed for 3 h. The yellow solution was distilled, and the residue was recrystallized from benzene to give **(3)**. Yield, 71% (benzene), m.p. 202 °C - 203 °C; IR (KBr), $\nu_{\max}$ = 3116 (NH), 1732 (lactone C=O), 1680 (OCN), 1352 cm^{-1} (CS); $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.62 (1H, s, NH), 8.12 (1H, s, C₅-H), 7.22 - 7.98 (10H, m, Ar H), 6.76 (2H, s, NH₂). Elemental Analysis (%) Calcd for C₁₉H₁₇N₇O₂S₂ (439.38): C, 51.94; H, 3.90; N, 22.31. Found: C, 51.22; H, 3.77; N, 22.46.

2-((5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)methylene)-N-phenylhydrazinecarbothioamide (4). A solution of compound **1** (1 g) in ethanol (30 ml) was treated with phenyl thiosemicarbazide (1 g) and a few drops of acetic acid. The reaction mixture was boiled under reflux for 4 h, concentrated, cooled, and the solid that separated was filtered off, successively washed with water and ethanol, and dried (yield 0.5 g). It was recrystallized from ethanol in yellow needles, Yield, 80 %, m.p. 210 °C - 211 °C; IR (KBr), $\nu_{\max}$ = 3116 (NH), 1732 (lactone C=O), 1680 (OCN), 1352 cm^{-1} (C=S); $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.62 (1H, s, NH), 8.12 (1H, s, C₅-H), 7.22 - 7.98 (10H, m, Ar H), 6.76 (2H, s, NH₂). Elemental Analysis (%) Calcd for C₂₃H₁₉N₇OS (441.14): C, 62.57; H, 4.34; N, 22.21. Found: C, 62.22; H, 4.43; N, 22.64.

4-methyl-6-oxo-3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-N-phenylpyridazine-1(6H)-carbothioamide (5). A solution of compound **4** (1g) in ethanol (30 ml) was refluxed with ethyl acetoacetate (1 g) for about 3 - 5 h. The reaction mixture was concentrated and cooled. The solids

that separated were filtered off, successively washed with water and ethanol, and dried (yield 0.5 g). It was recrystallized from ethanol as red needles. m.p. 262°C - 263°C. IR (KBr), $\nu_{\max}$ = 3116 (NH), 1712 (ester), 1680 (OCN), and 1260 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO-d_6) = 13.64 (1H, s, NH), 7.32 - 8.1 (15H, m, ArH), and 2.0 (3H, s, CH_3). Elemental analysis (%) Calcd for $\text{C}_{27}\text{H}_{21}\text{N}_7\text{O}_2\text{S}$ (507.49): C, 63.89; H, 4.19; N, 19.32. Found: C, 63.68; H, 4.32; N, 19.06.

(1E)-5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazole-3-carbaldehyde oxime (6a). A solution of compound 1 (1 g) in ethanol (30 ml) was treated with hydroxylamine hydrochloride (1 g), sodium acetate (1 g), and a few drops of acetic acid. The reaction mixture was boiled under reflux for 2 h, concentrated, and cooled, and the solid that separated was filtered off, successively washed with water and ethanol, and dried (yield 0.5 g). It was recrystallized from ethanol in yellow needles, m.p. 218°C - 219°C. IR (KBr), $\nu_{\max}$ = 3350 (OH), 1730 (lactone C=O), 1600 cm^{-1} (C=N). Elemental Analysis (%) Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_2$ (309): C, 62.53; H, 4.26; N, 22.79. Found: C, 62.38; H, 4.71; N, 22.73.

3-(hydrazonomethyl)-1-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (6b). A mixture of 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde (**1**) (0.3 g; 1.35 mmol) in absolute ethanol (20 ml) was treated with an equimolar amount of hydrazine hydrate in the presence of acetic acid. The reaction mixture was boiled under reflux for 2 hours, concentrated, and cooled. The solid that separated was filtered off, successively washed with water and ethanol, and dried (yield: 0.5 g). It was recrystallized from ethanol into yellow needles, m.p. 208°C - 209°C. IR (KBr), $\nu_{\max}$ = 3400 (NH), 1730 (lactone C=O), 1600 cm^{-1} (C=N). Elemental Analysis (%) Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_6\text{O}$ (306): C, 62.74; H, 4.61; N, 27.44. Found: C, 62.38; H, 4.71; N, 27.30.

3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-1,4,2-oxathiazinan-6-one (7). A mixture of compound (**6a**) (0.1 g, 0.3 mmol) and 2-mercaptoacetic acid (0.46 g, 5 mmol) was stirred in absolute ethanol (30 mL) for approximately 15 min, and then refluxed for 3 h. The yellow solution was distilled, and the residue was recrystallized from ethanol to give (**7**) as orange needles. Yield, 71%, m.p. 253°C - 254°C; IR (KBr), $\nu_{\max}$ = 3122 (NH), 1732 (lactone C=O), 1680 (OCN), and 1600 cm^{-1} (C=N). Elemental Analysis (%) Calcd for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{O}_3\text{S}$ (409.6): C, 66.69; H, 3.96; N, 18.37. Found: C, 66.48; H, 3.79; N, 18.12.

2,6-diphenyl-2H-pyrazolo[4,3-d][1,2,3]triazin-7(6H)-one (8).

2,6-diphenyl-2H-pyrazolo[4,3-d][1,2,3]triazine-7(6H)-one was prepared according to the literature procedure as red needles, m.p. 228°C - 229°C; Lit. m.p. 228°C - 230°C [27], IR (KBr), $\nu_{\max}$ = 1732 (lactone C=O), 1680 (OCN), 1600 cm^{-1} (CN), no NH absorption band; $^1\text{H-NMR}$ (DMSO-d_6) δ (ppm) = 8.84 (1H, s, C-H), 7.32 - 8.12 (10H, m, Ar H). Elemental Analysis (%) Calcd for $\text{C}_{16}\text{H}_{11}\text{N}_5\text{O}$ (289.10): C, 66.43; H, 3.83; N, 24.21. Found: C, 66.48; H, 3.73; N, 24.52.

3-(4,6-dimethylpyridazin-3-yl)-1-methyl-4-(2-phenylhydrazono)-1H-pyrazol-5(4H)-one (9)

A solution of compound **6b** (1 g) in ethanol (30 ml) was refluxed with acetylacetone (1 g) in the presence of a few drops of acetic acid for about 3 - 5 h. The reaction mixture was concentrated and cooled. The solids that separated were filtered off, successively washed with water and ethanol, and dried (yield 0.5 g). It was recrystallized from ethanol as red needles. m.p. 255°C - 256°C. IR (KBr), $\nu_{\max}$ = 3170 (NH), 1668 (OCN), and 1600 cm^{-1} (C=N). $^1\text{H-NMR}$ (DMSO- d_6) = 8.06 (1H, s, NH of the hydrazone moiety, 7.48 - 8.11 (10H, m, Ar H) moiety), 2.0 (3H, s, CH_3) and 1.98 (3H, s, CH_3). Elemental Analysis (%) Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_6\text{O}$ (370.40): C, 68.09; H, 4.90; N, 22.69. Found: C, 68.21; H, 4.78; N, 22.73.

6-methyl-3-(1-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl) pyridazin-4(5H)-one (10). A solution of compound **6b** (1 g) in ethanol (30 ml) was refluxed with ethylacetoacetate (1 g) in the presence of a few drops of acetic acid for about 3 - 5 h. The reaction mixture was concentrated and cooled. The solids that separated were filtered off, successively washed with water and ethanol, and dried (yield 0.5 g). It was recrystallized from ethanol as red needles. m.p. 166°C - 167°C. IR (KBr), $\nu_{\max}$ = 3320 (OH), 3116 (NH), 1680 (OCN), and 1600 cm^{-1} (C=N). $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.22 (1H, s, NH-hydrazone), 7.32 - 8.02 (10H, m, Ar H) moiety), 2.0 (3H, s, CH_3) and 1.98 (3H, s, CH_3). Elemental Analysis (%) Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2$ (372.38): C, 64.51; H, 4.33; N, 22.57. Found: C, 64.38; H, 4.16; N, 22.70.

3-amino-6-(1-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (11).

Thiosemicarbazide (1 mmol) was added dropwise to an ethanolic solution of the pyrazole aldehyde (**1**) (1 mmol), and stirred for 30 min; then, 2-ethyl cyanoacetate (1 mmol) was added dropwise to this mixture, and a few drops of triethylamine. The mixture was stirred well for the required time. The clear solution was heated under reflux for 2 h. The reaction process was monitored by TLC (n-hexane: ethyl acetate (6:3)). After completion of the reaction, the solution was kept at room temperature for evaporation to dryness, then recrystallized from ethanol in red needles. m.p. 225°C - 226°C. IR (KBr), $\nu_{\max}$ = 3268 (NH), 2222 ($\text{C}\equiv\text{N}$), 1680 (OCN), 1600 (CN), and 1251 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.26 (1H, s, $\text{N}_1\text{-H}$ of the pyrimidine ring), 8.32 (1H, s, $\text{N}_3\text{-H}$ of the pyrimidine ring), 7.32 - 8.21 (11H, m, Ar H, NH of the hydrazone moiety), and 6.34 (2H, br s, NH_2). Elemental analysis (%) Calcd for $\text{C}_{20}\text{H}_{14}\text{N}_8\text{O}_2\text{S}$ (430.36): C, 55.81; H, 3.28; N, 26.02. Found: C, 55.67; H, 3.40; N, 26.11.

4-oxo-6-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (12).

Thiourea (1 mmol) was added dropwise to an ethanolic solution of the pyrazole aldehyde (**1**) (1 mmol), and stirred for 30 min; then, 2-ethyl cyanoacetate (1 mmol) was added dropwise to this mixture and a few drops of triethylamine. The clear solution was heated under reflux for 2 h. The progress of the reaction was monitored by TLC (n-hexane: ethyl acetate (6:3)). After completion of the reaction, the solution was kept at room temperature for evaporation to dryness. The solid obtained was filtered off, washed with cold water and ethanol, and dried. The

product was recrystallized from ethanol in orange needles. m.p. 176°C - 177°C. IR (KBr), $\nu_{\max}$ = 3226 (NH), 2222 ($\text{C}\equiv\text{N}$), 1680 (OCN), 1600 (CN), and 1251 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO- d_6) = 13.76 (1H, s, N_1 -H of the pyrimidine ring), 8.00 (1H, s, N_3 -H of the pyrimidine ring), and 6.81 - 7.94 (11H, m, Ar H, NH of the hydrazone moiety). Elemental Analysis (%) Calcd for $\text{C}_{20}\text{H}_{13}\text{N}_7\text{O}_2\text{S}$ (415.43): C, 57.82; H, 3.15; N, 23.60. Found: C, 57.70; H, 3.34; N, 23.32.

Ethyl 7-oxo-2,6-diphenyl-6,7-dihydro-2H-pyrazolo[3,4-e] [1,2,4] triazine-3-carboxylate (13).

A solution of compound (1) (1 g) in ethanol (30 ml) was treated with 2-ethylcyanoacetate (1 g) and a few drops of triethylamine. The reaction mixture was boiled under reflux for 2 hours, concentrated, and cooled. The solid that separated was filtered off, successively washed with water and ethanol, and dried (yield: 0.5 g). It was recrystallized from ethanol in orange needles, m.p. 208°C - 209°C. IR (KBr), $\nu_{\max}$ = no (NH) band, 1738 (ester C=O), 1678 (OCN) and 1595 cm^{-1} (C=N). $^1\text{H-NMR}$ (DMSO- d_6) = 7.44 - 8.0 (10H, m, Ar H), 4.14 (2H, q, CH_2) and 1.84 (3H, t, CH_3). Elemental Analysis (%) Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_3$ (360.36): C, 66.66; H, 4.48; N, 15.55. Found: C, 66.48; H, 4.69; N, 15.22.

Ethyl-6-methyl-4-(1-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (14).

A solution of 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde (1) (0.3 g; 1.35 mmole) in absolute ethanol (20 ml) was treated with an equimolar amount of ethyl acetoacetate, thiourea, and a few drops of triethylamine. The clear solution was heated under reflux for 3 h, concentrated, poured onto ice water, and neutralized with acetic acid. The solid obtained was filtered off, washed with cold water, ethanol, and dried. The product was recrystallized from ethanol in red needles. m.p. 240°C - 241°C. IR (KBr), $\nu_{\max}$ = 3104 (NH), 1692 (ester), 1669 (OCN), and 1245 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.21 (1H, s, N_1 -H of the pyrimidine ring), 8.32 (1H, s, N_3 -H of the pyrimidine ring), 7.02 - 8.08 (11H, m, Ar H, NH of the hydrazone moiety), 8.05 (1H, s, C_5 -H), 4.55 (2H, q, CH_2), 2.41 (3H, s, CH_3) and 1.52 (3H, t, CH_3). Elemental Analysis (%) Calcd for $\text{C}_{23}\text{H}_{22}\text{N}_6\text{O}_3\text{S}$ (462.45): C, 59.73; H, 4.80; N, 13.17. Found: C, 59.53; H, 4.62; N, 13.36.

Ethyl-1-amino-6-methyl-4-(1-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (15)

A solution of compound (1) (0.3 g; 1.35 mmol) in absolute ethanol (20 ml) was treated with an equimolar amount of ethyl acetoacetate, thiosemicarbazide, and a few drops of triethylamine. The clear solution was heated under reflux for 3 h, concentrated, poured onto ice water, and neutralized with acetic acid. The solid obtained was filtered off, washed with cold water, ethanol, and dried. The product was recrystallized from ethanol into red needles. m.p. 235°C - 236°C. IR (KBr), $\nu_{\max}$ = 3104 (NH), 1692 (ester), 1669 (OCN), and 1245 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.21 (1H, s, N_1 -H of the pyrimidine ring), 8.32 (1H, s, N_3 -H of the pyrimidine ring), 8.05 (1H, s, C_5 -H), 7.02 - 8.08 (11H, m, Ar H, NH of

the hydrazone moiety) 4.55 (2H, q, CH₂), 2.41 (3H, s, CH₃), and 1.52 (3H, t, CH₃). Elemental Analysis (%) Calcd for C₂₃H₂₃N₇O₃S (462.45): C, 59.73; H, 4.80; N, 13.17. Found: C, 59.53; H, 4.62; N, 18.36.

(4E)-2-methyl-4-((1-methyl-5-oxo-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)methylene)oxazol-5(4H)-one (16)

An equimolar mixture of compound (1) (0.3 g; 1.00 mmol) and ethyl 2-acetamidoacetate (1.00 mmol) in the presence of anhydrous sodium acetate and acetic anhydride was refluxed at 80 °C for 3 - 5 h. The reaction mixture was poured into crushed ice with stirring. The solid that separated was filtered off, successively washed with water, ethanol, and dried. The product was recrystallized from ethanol in red needles. m.p. 254 °C - 255 °C. IR (KBr), $\nu_{\max}$ = 3218 (NH), 1732 (lactone CO), 1680 (OCN), and 1600 cm⁻¹ (CN). ¹H-NMR (DMSO-d₆) δ (ppm) = 13.22 (1H, s, NH), 7.38 - 8.16 (10H, m, Ar H), 8.02 (1H, s, C₅-H) and 1.98 (3H, s, CH₃). Elemental Analysis (%) Calcd for C₂₀H₁₅N₅O₃ (373.36): C, 64.33; H, 4.05; N, 18.76. Found: C, 64.36; H, 3.18; N, 18.58.

2.4. Structural Characterization

All the compounds synthesized were characterized by IR, ¹H-NMR, and elemental analysis. Successful cyclization reactions and the incorporation of heteroatoms were confirmed by characteristic diagnostic peaks. Spectral peaks were used to confirm the formation of rings, patterns of substitution, and the presence or absence of NH, C=O, C=N, C=S, and functional groups of a lactone [51] [52].

The suggested reaction pathways were confirmed by the disappearance of the typical aldehyde bands and the appearance of new heterocyclic signals wherever needed.

2.5. Reproducibility, Quality Control, and Experimental Reliability

All the experimental procedures were performed under standardized and controlled conditions to make the synthetic and biological results reproducible and robust. The preliminary experiments conducted on reaction conditions, including solvent selection, temperature, reaction time, and molar ratios, were optimized before the scale-up synthesis. All the compounds were prepared on at least two occasions to ensure consistent yield, purity, and spectral data.

Assessment of purity was conducted through thin-layer chromatography with suitable solvent systems, and compounds that exhibited more than one spot were further purified until only one distinct spot was observed. Determinations of melting points were another measure of the purity of the compound and the reproducibility from batch to batch.

All IR and NMR spectra were characterized by various researchers to minimize assignment bias in spectroscopic characterization. Elemental analysis values had to be within ± 0.4 percent of the calculated theoretical values, which were within acceptable analytical limits. Compounds that did not fulfill this condition were reformulated and recharacterized.

All antimicrobial and antioxidant studies were conducted three times in biological assays, and the mean values were presented to minimize random error. Every run of the experiment had positive and negative controls to validate the assay performance. Solvent controls were also used to ensure that DMSO at the concentrations used did not affect the growth or radical scavenging of the microbes.

Antioxidant data were subjected to statistical analysis in order to determine IC_{50} properly. The non-linear regression models were used, which have proven to provide more reliable estimates of the activity than the linear interpolation models. All of these quality-control measures will make the experimental results more credible and support the validity of the reported structure-activity relationships.

2.6. Antimicrobial Assay

Antimicrobial testing was conducted using the agar well-diffusion method. Compounds were screened against:

- *Staphylococcus aureus* (ATCC 25923)
- *Escherichia coli* (ATCC 8739)
- *Candida albicans* (ATCC 10231)

Inoculum preparation, agar preparation, well formation, sample loading, incubation, and zone-measurement procedures followed the detailed protocol already presented in the manuscript. Compounds were dissolved in DMSO (1 mg/mL), and levofloxacin and natamycin served as positive controls. Zones of inhibition were measured in millimeters after 24 h of incubation at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

2.7. Antioxidant Assay (DPPH)

The radical-scavenging ability of selected synthesized compounds (5, 11, 12, 13, 14 and 16) as shown in **Figure 8**, was evaluated using the DPPH assay. Trolox was used as the reference antioxidant [53]. Absorbance was measured at 540 nm after 30 min of incubation in the dark.

$$\%RSA = (A_0 - A_t)/A_0 \times 100$$

All measurements were performed in triplicate, and blank controls were included on every plate.

2.8. Data Analysis

- IC_{50} values were calculated from dose-response curves.
- Data normalization and statistical evaluation were performed using Microsoft Excel and GraphPad Prism 9.
- IC_{50} values were derived from non-linear regression analysis using the log(inhibitor) vs. normalized response model, and the results are reported as mean $\pm$ standard error.

The 4PL equation for DPPH inhibition curves is:

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + \left(\frac{X}{\text{IC}_{50}} \right)^{\text{Hillslope}}}$$

where:

Y = % inhibition

X = concentration (mg/mL)

Top $\approx$ maximum inhibition plateau

Bottom $\approx$ baseline inhibition

HillSlope $\approx$ curve steepness

IC_{50} = concentration giving 50% inhibition

3. Results

3.1. Chemistry Outcomes

Our designed pathways to produce the targeted compounds depend on using fewer reaction steps in our methodologies. As shown in **Figure 2**, the thiadiazinone derivative (3) was synthesized through a two-step process. Firstly, the reaction of the pyrazole carbaldehyde (1) with thiosemicarbazide gives the corresponding thiosemicarbazone derivative (2). When treated with thioglycolic acid, it yields a new and unexpected thiadiazinone derivative (3) instead of 1-(4-oxo-2-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl) thiazolidin-3-yl)thiourea.

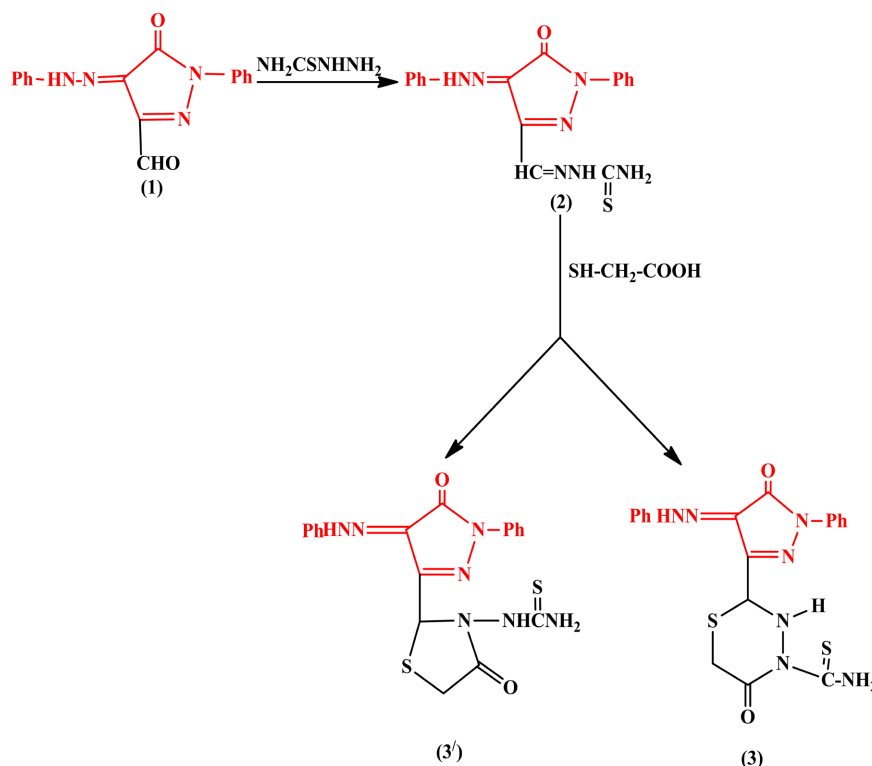


Figure 2. Synthesis of 1,3,4-thiadiazin-5-one.

The formation of compound (3) was achieved via the nucleophilic addition of the sulfur atom to the activated C=N, to give a non-isolable addition product which cyclizes give thiadiazinone derivative (3). The structure of the thiadiazinone (3) was confirmed by elemental analysis, IR, and ¹H-NMR spectra. The infra-red spectra showed the presence of 3116 (NH), 1732 (lactone C=O), 1680 (OCN), 1352 cm⁻¹ (CS); ¹H-NMR (DMSO-d₆) δ (ppm) = 13.62 (1H, s, NH), 8.12 (1H, s, C5-H), 7.22 - 7.98 (10H, m, Ar H), 6.76 (2H, s, NH₂). Also, elemental analysis confirmed the predicted structure. Similarly, the reaction of the starting compound (1) with phenyl thiosemicarbazide gave the corresponding condensation products (4), which, when treated with thioglycolic acid, give a new and unexpected 4-methyl-6-oxo-3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-N-phenyl pyridazine-1(6H)-carbothioamide (5). **Figure 3**, which was confirmed by elemental analysis, IR, and ¹H-NMR spectra.

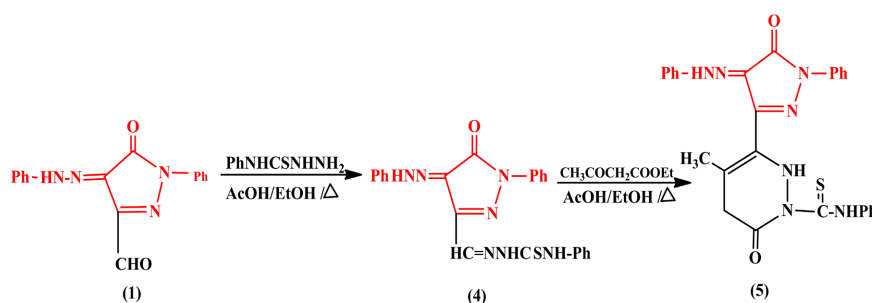


Figure 3. Synthesis of N-phenylpyridazine-6-one (5).

On the other hand, treatment of (1) with hydroxylamine or hydrazine hydrate gave the corresponding oxime (6a) or the hydrazone (6b), respectively. The reaction of (6a) with thioglycolic acid gave 3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4,5-dihydro-1H-pyrazol-3-yl)-1,4,2-oxathiazinan-6-one (7). The infrared spectrum of (7) showed peaks at 3122 cm⁻¹ (NH), 1732 cm⁻¹ (lactone C=O), 1680 cm⁻¹ (OCN), and 1600 cm⁻¹ (C=N). Elemental analysis confirmed its structure. Novel synthetic approaches involving multiple N–N, N–O, and N–S bond formations that afford biologically active N-heterocycles are in high demand. The Cu-catalyzed direct C–H bond functionalization for preparing complex molecules [43], particularly for carbon–carbon and carbon–heteroatom (nitrogen, oxygen, and sulfur) bond formation, has received our attention. Accordingly, the reaction of (6a) with cuprous chloride led to the formation of the diphenyl-pyrazolo triazinone (8). Its infrared spectrum showed the disappearance of the NH absorption band, which confirms the expected product. Treatment of compound (6b) in ethanol, with acetylacetone in the presence of acetic acid, gave the corresponding 4,6-dimethylpyridazin phenyl hydrazono pyrazolone (9), whereas its reaction with ethyl acetoacetate gave the pyridazinone derivative (10). **Figure 4**. The structures of (9) & (10) were confirmed using different spectral tools.

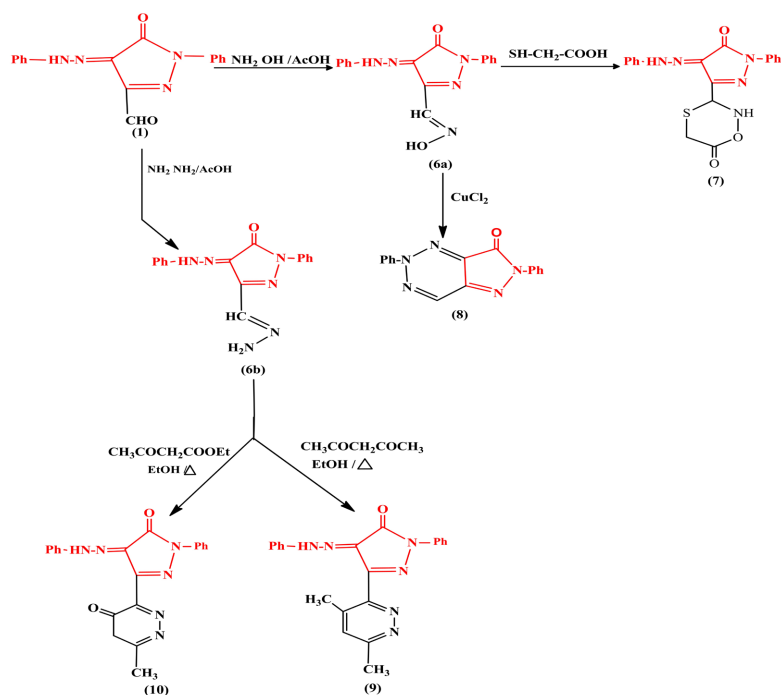


Figure 4. Synthesis of new heteroarenes (6–10).

One-pot synthesis of the binary heterocyclic compound (11), (12) takes place according to the simulation of C. O. Kappe's proposed mechanism [44] (**Figure 5**).

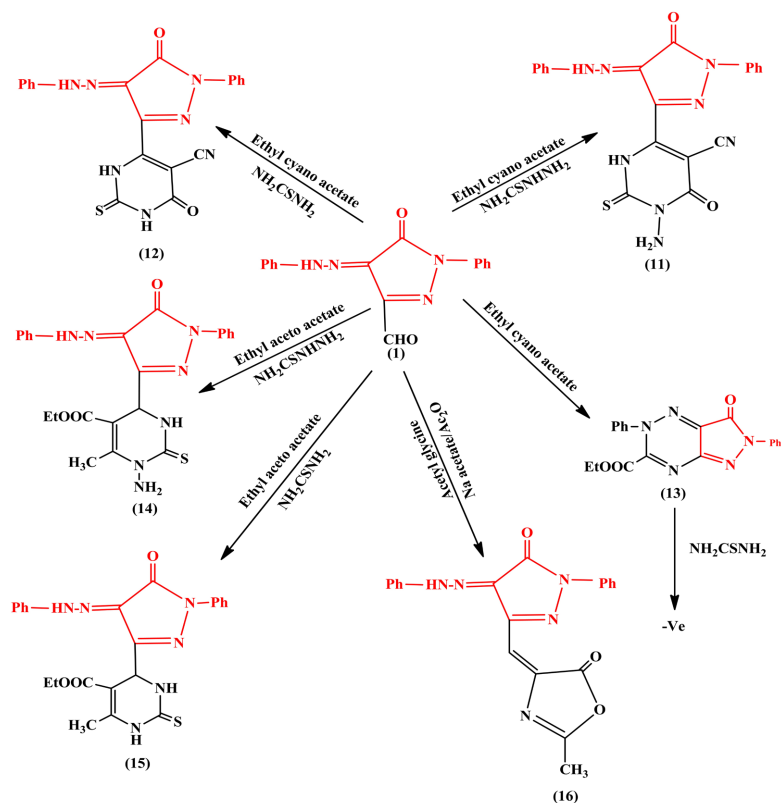


Figure 5. Synthesis of some new heterocyclic compounds (11–16).

The reaction of the pyrazole aldehyde (**1**) with ethyl cyanoacetate and thiosemicarbazide (**11**) or thiourea (**12**), respectively, is shown as follows:

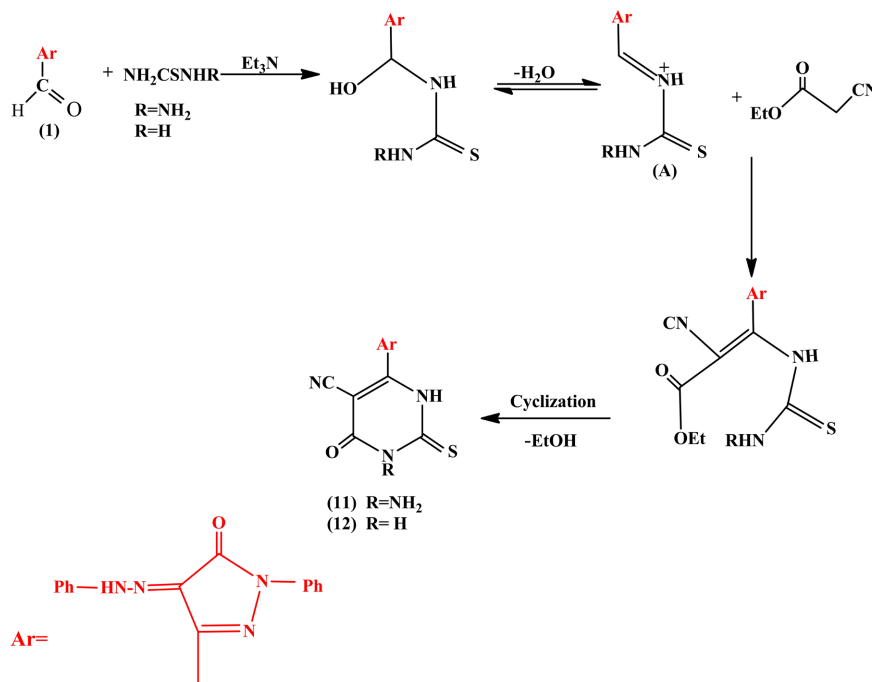


Figure 6. Mechanistic pathway for the synthesis of (**11**) & (**12**).

The first addition step of the aldehyde with thiosemicarbazide or thiourea gives the adduct (**A**). The final desired product (**11**, **12**) was observed by the addition of ethyl cyanoacetate to the iminium ion (**A**), followed by cyclization via the elimination of EtOH (**Figure 6**). IR spectra showed $\nu_{\max}$ = 3268 (NH), 2222 ($\text{C}\equiv\text{N}$), 1680 (OCN), 1600 (CN), and 1251 cm^{-1} (C=S). $^1\text{H-NMR}$ (DMSO- d_6) δ (ppm) = 13.26 (1H, s, $\text{N}_1\text{-H}$ of the pyrimidine ring), 8.32 (1H, s, $\text{N}_3\text{-H}$ of the pyrimidine ring), 7.32 - 8.21 (11H, m, Ar H, NH of the hydrazone moiety), and 6.34 (2H, br s, NH_2). Continuously, the reaction products were confirmed when the pyrazole aldehyde (**1**) reacted with ethyl cyanoacetate, which cyclized to give (**13**). When (**13**) was reacted with thiourea, the unchanged product was obtained. IR spectra showed $\nu_{\max}$ no (NH) band, 1738 (ester $\text{C}=\text{O}$), 1678 (OCN), and 1595 cm^{-1} ($\text{C}=\text{N}$). $^1\text{H-NMR}$ (DMSO- d_6) = 7.44 - 8.0 (10H, m, Ar H), 4.14 (2H, q, CH_2), and 1.84 (3H, t, CH_3).

This data was in agreement with the proposed mechanism in **Figure 6**.

The formation of the pyrimidine derivatives (**14**), (**15**) depends on the aldol condensation of pyrazole aldehyde (**1**) with β -ketoester in the presence of triethylamine, leading to the unsaturated ketoester (**A**), which reacts with thiourea or thiosemicarbazide to give the Michael addition product (**B**), undergoes cyclization, and affords the desired dihydropyrimidines [45] (**Figure 7**).

Finally, the reaction of pyrazole aldehyde (**1**) with ethyl 2-acetamidoacetate in the presence of anhydrous sodium acetate and acetic anhydride afforded the cor-

responding oxazolone derivative (16), as shown in **Figure 5**. The structure of (16) was confirmed using various spectral tools, as shown in the experimental section.

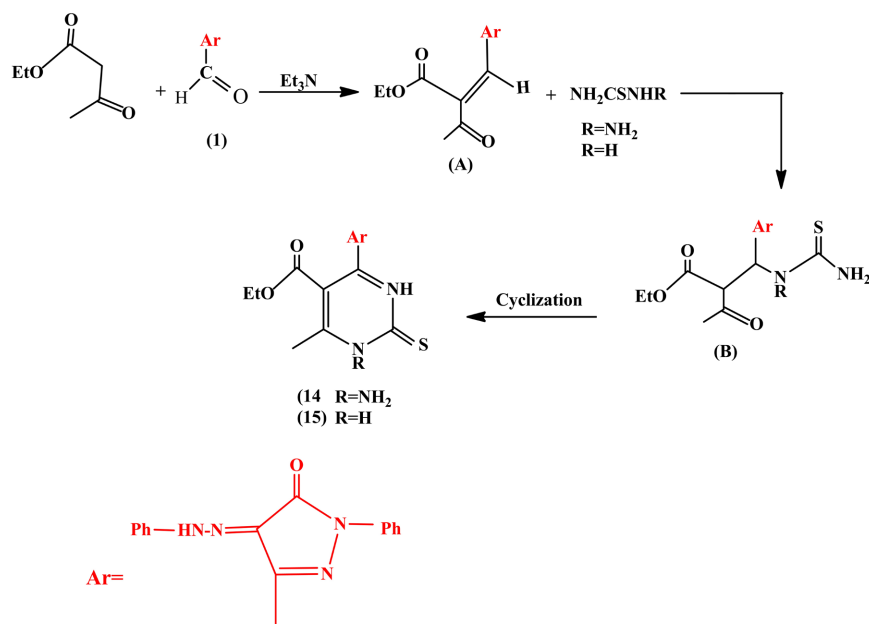


Figure 7. Mechanistic pathway for the synthesis of (14) & (15).

3.2. Spectral Confirmation

Elemental analysis, IR, and the use of ^1H -NMR methods were used to elucidate the structure. The expected functionalities were verified by diagnostic absorption bands and chemical shifts. Typical IR absorptions were NH ($\approx 3100 - 3300\text{ cm}^{-1}$) stretches, C=O ($\approx 1710 - 1740\text{ cm}^{-1}$) lactone/ester, C=N ($\approx 1600\text{ cm}^{-1}$), and C=S ($\approx 1240 - 1350\text{ cm}^{-1}$). The loss of aldehydic C=O bands and the emergence of novel heterocyclic signatures supported the events of the C=O bond closure.

The singlets in the ^1H -NMR spectra were those of NH protons of hydrazone and pyrimidine groups, aromatic multiplets ($\approx 7.0 - 8.2\text{ ppm}$), and singlets of methyl groups where possible. The success of the synthesis of the target heterocycles was conclusively validated using these data and confirmed pathways to heteroatom incorporation and cyclization. Spectral patterns also matched analogues reported previously, with structural assignments again being reinforced in a number of cases.

3.3. Antimicrobial Activity

The antimicrobial activity of compounds (3), (5), (11), (12), (13), (14), and (16) as shown in **Figure 8**, was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the agar well-diffusion technique.

Inhibition areas are summarized in **Table 1**. There was no significant antibacterial action against Gram-positive and Gram-negative strains. Nevertheless, every compound tested proved to be active against *Candida albicans*, and the inhibition zones of all of them were moderate to strong.

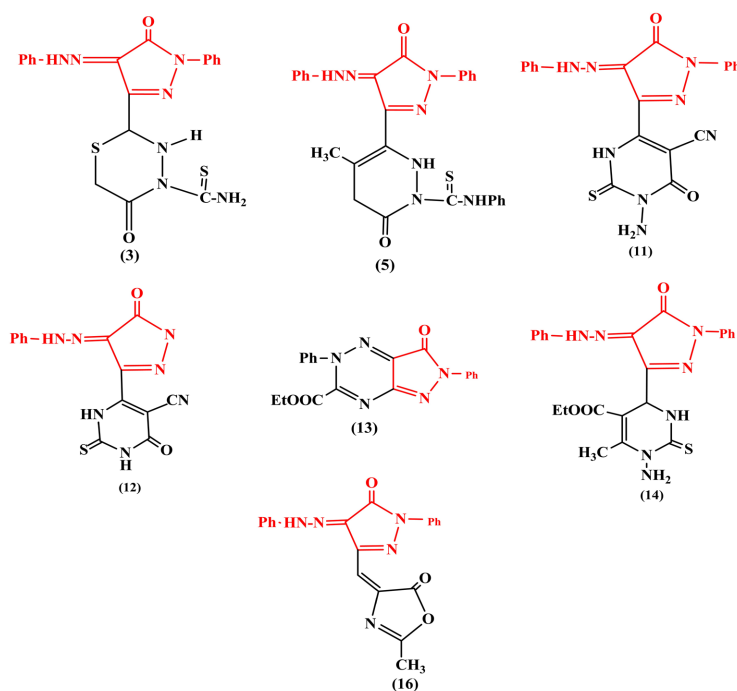


Figure 8. Moderate zone of inhibition against the tested fungal strains.

Table 1. Antimicrobial activity of the tested synthesized compounds.

Tested Strain (Reference)	+ve Control	-ve Control	3	5	11	12	13	14	16
Staphylococcus aureus (MRSA) ATCC 25923	35 mm	-	-	-	-	-	-	-	-
Escherichia coli (E. coli) ATCC 8739	38 mm	-	-	-	-	-	-	-	-
Candida albicans ATCC 10231	25 mm	15 mm	17 mm	19.5 mm	16.25 mm	21 mm	18.5 mm	15 mm	21.5 mm

Note: (-) no inhibition zone.

Compounds (12) and (16) were found to have significant anti-fungal activity, as they gave the largest zones in comparison with other derivatives that were produced. Compounds (13), (5), (11), and (3) were of medium activity. To illustrate comparative inhibition, **Figure 9** presents an antifungal activity chart.

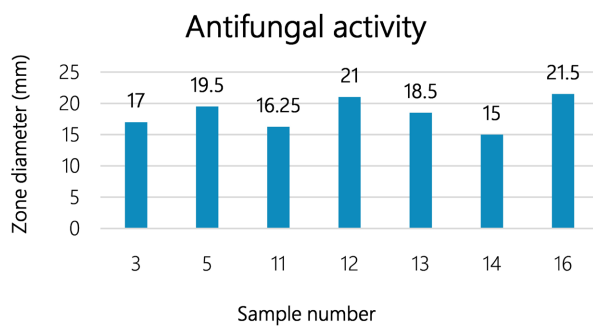


Figure 9. Antifungal activity of some newly synthesized heterocyclic compounds.

These results suggest that **(12)** and **(16)** have structural features that may confer selective antifungal activity, possibly attributable to interactions with fungal membranes or enzymes.

3.4. Antioxidant Performance

The antioxidant activity of the test samples was evaluated using the DPPH radical-scavenging assay, with Trolox employed as a reference standard. Data analysis was normalized using Microsoft Excel and the IC_{50} value was calculated using GraphPad Prism 9. The concentrations were converted into logarithmic values, and a non-linear inhibitor regression equation: (log (inhibitor) vs. normalized response variable slope equation) was selected.

Linearity of Trolox in the DPPH assay

The DPPH-reducing ability of the samples is presented as $\mu\text{M TE/mg sample}$ using the linear regression equation extracted from the following calibration curve (linear dose-response curve of Trolox).

Table 2. The Trolox calibration curve data.

Concentration (μM)	Inhibition
3.90625	2.80
7.8125	4.28
15.625	10.70
31.25	21.80
62.5	51.83
100	78.91

The Trolox calibration curve (3.9 - 100 (μM)) exhibited a sigmoidal dose-response, reaching a maximum inhibition of $\sim 78.91\%$ at 100 (μM). Nonlinear regression analysis yielded an IC_{50} value of 62.5 (μM), consistent with the reported antioxidant potency of Trolox. **Table 2.**

Figure 10(a) represents the average of the readings of the 3 replicates that were taken after subtraction from the Blank (DPPH reagent + solvent).

The DPPH-reducing ability of the samples is presented as $\mu\text{M TE/mg sample}$ using the linear regression equation extracted from the following calibration curve, **Figure 10(b)**). Recalculate using blank-corrected absorbances:

$$A_{\text{sample, corrected}} = A_{\text{sample+DPPH, measured}} - A_{\text{sample, blank}}$$

Then

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample, corrected}}}{A_{\text{control}}} \times 100 \quad (1)$$

The DPPH radical-scavenging assay was used to determine antioxidant activity. **Table 3** represents the results of compounds **(3)**, **(5)**, **(11)**, **(12)**, **(13)**, **(14)**, and **(16)**, with the concentration-dependent results in radical scavenging activity (RSA) percentage.

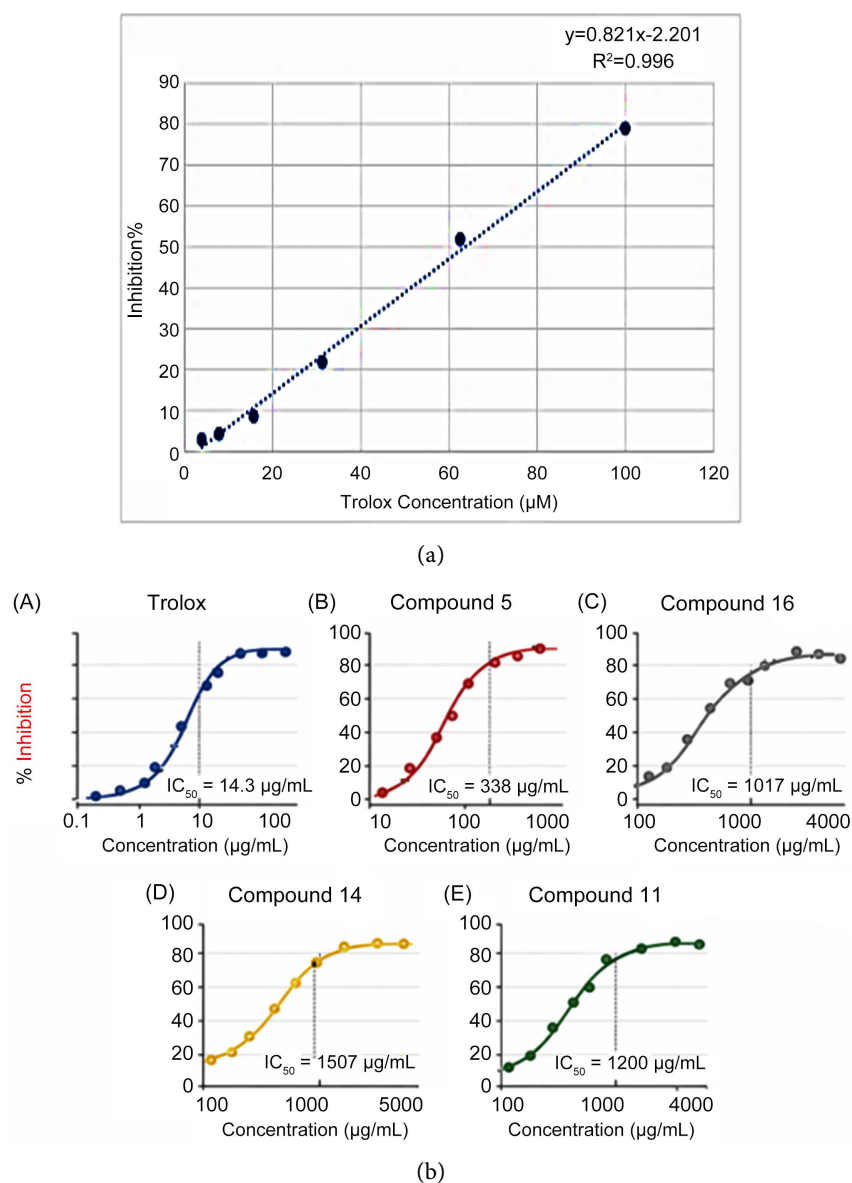


Figure 10. (a) Trolox calibration curve; (b) Dose-response curves for Trolox and synthesized heterocycles.

Table 3. Radical scavenging activity of the compound under investigation.

Sample ID	Applying Equation (1), the % of inhibition	Substitution in cal. Curve equation (μM)	Micromole Trolox equivalent per mg sample ($\mu\text{M TE/mg sample}$)	Standard deviation
3	21.619	29.013	290.133	11.667
5	21.267	28.585	285.846	15.910
11	15.487	21.545	68.943	4.509
12	6.109	10.121	101.213	1.299
13	6.637	10.764	107.643	7.794
14	14.155	19.922	63.750	3.743
16	22.953	30.638	98.043	2.623

The maximum soluble concentrations of samples (5), (11), (14), and (16) showed inhibition ratios above 50%, so we can calculate their IC₅₀ values. **Table 4.** Whereas samples (3), (12), and (13) showed inhibition ratios below 50%, so we did not calculate their IC₅₀ values and reported them as equivalent values only.

- **Trolox** shows a steep, sigmoidal increase in inhibition with concentration — typical of a potent radical scavenger.
- **Compound (5)** exhibits a slower rise, reaching only ~58% inhibition at 400 mg/mL, suggesting moderate activity.
- **Compounds (16), (14), (11)** show gradual increases but require much higher concentrations to approach 50% - 70% inhibition, indicating **lower antioxidant efficiency**.
- The overall potency order is: **Trolox** >> (5) > (11) > (16) > (14).
- The higher IC₅₀ values for (5), (11), (16), and (14) mean they require larger doses to achieve 50% radical inhibition — hence weaker antioxidants.

Table 4. Calculation of IC₅₀ of standard Trolox, (5), (11), (14), and (16).

Sample	Conc (mg·mL ⁻¹)	Mean % inhibition	Approximate IC ₅₀ Trend
Standard Trolox	3.90625	8.62	≈15 mg/mL
	7.8125	21.80	
	15.625	51.84	
	25	78.91	
	31.25	85.23	
5	25	10.46	≈340 mg/mL
	50	14.38	
	100	21.27	
	200	33.18	
	400	57.37	
11	156.25	9.08	≈1200 mg/mL
	312.5	15.49	
	625	23.81	
	1250	41.60	
	2000	61.39	
14	312.5	7.63	≈1500 mg/mL
	625	14.16	
	1250	27.95	
	2000	46.55	
	2500	52.89	
16	156.25	11.44	≈1000 mg/mL
	312.5	22.95	
	625	36.72	
	1250	54.84	
	2000	68.06	

- Structural factors likely contribute: Trolox's phenolic hydroxyl and chromanol ring stabilize radicals efficiently, while your synthesized heterocycles may have fewer electron-donating groups or less resonance stabilization.
- Compound **5**'s moderate activity suggests partial retention of phenolic character or conjugation, whereas **(16)**, **(14)**, and **(11)** may have bulkier or less conjugated substituents that hinder radical quenching.

Dose–response fitting using a four-parameter logistic model revealed IC_{50} values of 14.3 $\mu\text{g/mL}$ for Trolox, 338 $\mu\text{g/mL}$ for compound **5**, and 1017 - 1507 $\mu\text{g/mL}$ for compounds **(16)**, **(14)**, and **(11)**. The results confirm **Trolox** as the most potent antioxidant, while compound **5** exhibits moderate activity, and the remaining derivatives show weak radical-scavenging efficiency.

The fitted 4PL curves in **Figure 10(b)** confirm **Trolox** as the most potent antioxidant, with compound **5** showing moderate activity and compounds **(16)**, **(14)**, and **(11)** exhibiting weak radical-scavenging efficiency. The IC_{50} values derived from these curves (14.3, 338, 1017, 1507, and 1200 $\mu\text{g/mL}$, respectively) quantitatively support the potency ranking **Trolox** $\gg$ **5** $>$ **11** $>$ **16** $>$ **14**.

1) **Trolox**, $IC_{50} = 14.3 \mu\text{g/mL}$.

2) **Compound (5)**, $IC_{50} = 338 \mu\text{g/mL}$.

3) **Compound (16)**, $IC_{50} = 1017 \mu\text{g/mL}$.

4) **Compound (14)**, $IC_{50} = 1507 \mu\text{g/mL}$.

5) **Compound (11)**, $IC_{50} = 1200 \mu\text{g/mL}$.

All curves were fitted using a four-parameter logistic model. Potency ranking: **Trolox** $\gg$ **5** $>$ **11** $>$ **16** $>$ **14**.

Table 5. Estimated IC_{50} values for the tested compounds **(5)**, **(11)**, **(14)**, and **(16)**.

Sample	Concentration Range (mg/mL)	% Inhibition Range	Fitted IC_{50} ($\mu\text{g/mL}$)	Relative Potency
Trolox	3.9 - 31.25	8 $\rightarrow$ 85 %	$\approx 14.3 \pm 1.0$	Highest
5	25 - 400	9 $\rightarrow$ 58 %	$\approx 338 \pm 1.1$	Moderate
16	156 - 2000	10 $\rightarrow$ 69 %	$\approx 1017 \pm 1.0$	Weak
14	312 - 2500	7 $\rightarrow$ 53 %	$\approx 1507 \pm 1.0$	Very weak
11	156 - 000	8 $\rightarrow$ 65 %	$\approx 1200 \pm 1.0$	Weak-moderate

The DPPH radical-scavenging activity of Trolox and the synthesized heterocyclic derivatives **(5)**, **(16)**, **(14)**, and **(11)** was evaluated using a four-parameter logistic model as in **Table 5**.

Trolox exhibited a steep sigmoidal dose–response curve with an IC_{50} of 14.3 $\mu\text{g/mL}$, confirming its strong antioxidant potency. **Compound (5)** displayed a moderate slope and an IC_{50} of 338 $\mu\text{g/mL}$, indicating partial retention of radical-scavenging capacity. **Compounds (16)**, **(14)**, and **(11)** produced shallower curves with IC_{50} values of 1017, 1507, and 1200 $\mu\text{g/mL}$, respectively, reflecting weaker activity.

The overall potency ranking (**Trolox** $\gg$ **5** $>$ **11** $>$ **16** $>$ **14**) demonstrates an

inverse relationship between IC_{50} and antioxidant strength. These results highlight the structural dependence of radical-scavenging efficiency, where the presence of phenolic or conjugated moieties enhances activity, while bulkier heterocyclic substitutions diminish electron-donating capacity.

All in all, the increased substitution density and electron-donating capabilities were associated with better radical-scavenging capacity.

3.5. Integrated Interpretation

Together, these findings indicate that the pyrazolone-derived heterocycles can be used for dual biological activity, with antimicrobial and strong antioxidant activity.

Each domain of activity seems to be explained by separate structural patterns, which determine the structure–activity optimization.

4. Discussion

The current paper outlines the preparation and biological assay of some new heterocyclic products based on a pyrazolone backbone. The synthetic pathways applied were efficient and provided structurally differing products by cyclization, condensation, and multicomponent reactions [54]. The formation of the designed molecules was structurally confirmed by IR, 1H -NMR, and elemental analysis. The similarity in the predicted and observed spectral characteristics supports the validity of the suggested synthetic mechanisms.

4.1. Analysis of Chemical Results

The chemical findings will be interpreted as follows:

The make of thiadiazinone (3), pyridazinones (5, 9, 10), triazinone (8), pyrimidinones (11–15), and oxazolone (16) reveals that pyrazole carbaldehyde (1) is a versatile precursor, which can take up various modes of cyclization [55]. This synthetic compliance is beneficial in that it enables systematic exploration of chemical space around the pyrazolone skeleton.

The appearance of intermediates and the disappearance of the aldehyde signals in the reactions also prove the suggested reaction pathways. Such observations are in line with literature reports that suggest that the heterocycle generation of pyrazolone aldehydes is mainly driven by the formation of hydrazone and intramolecular nucleophilic attack.

4.2. Biological Implications of the Antimicrobial Results

None of the compounds produced were active against *Staphylococcus aureus* or *Escherichia coli*, but some of them had strong antifungal activity against *Candida albicans* [56] [57]. The largest inhibition zones were generated by compounds 12 and 16.

Such a selective antifungal effect suggests the potential of these structures to bind fungi-specific biochemical targets, including ergosterol biosynthesis or

membrane-bound proteins. Lack of antibacterial activity could indicate variations in the permeability barriers or affinity of targets in bacterial systems [58].

4.3. Behavior of Antioxidants and Structure-Activity Relationships

The concentration-dependent radical scavenging of all the tested compounds was shown by the DPPH results, with the highest values of radical-scavenging activity for compounds (5), (14) and (16).

Antioxidant properties were found to be stronger in compounds containing electron-donating substituted compounds and containing extended conjugated systems, presumably [59]. Conversely, molecules with these characteristics needed a higher concentration to reach the same inhibition, which is a sign of weak radical-quenching ability.

4.4. Integrated SAR Perspective

Combined, the results suggest that different structural modifications exert distinct effects on biological outputs.

- Fused or sulfur-based heterocycles—increased antifungal activity [60].
- Electron-Hex rich substituents and conjugation-enhanced antioxidant activity.

This dual-profile SAR demonstrates the tunability of the pyrazolone scaffold and provides a basis for future optimization.

4.5. Mechanistic Considerations for Antifungal and Antioxidant Activities

The selective antifungal activity of a number of the compounds produced indicates that there are selective interactions between the products and the fungal cellular architecture [61]. *Candida albicans* has unique structural and biochemical features, such as ergosterol-enriched membranes and fungal-specific enzymes, and can be selectively attacked by sulfur- and nitrogen-containing heterocycles. The compounds (12 and 16), which showed the highest antifungal activity, have functional groups that are capable of hydrogen bonding or are nucleophilic, which may bind to fungal membrane proteins or enzymes involved in cell wall construction.

The inability to produce antibacterial activity toward both Gram-positive and Gram-negative bacteria can be explained by cell wall structure and permeability barrier variations [62]. Gram-negative bacteria can often impede the uptake of large or highly polar molecules into bacterial outer membranes. Conversely, fungal cells can promote the uptake of lipophilic heterocyclic compounds, resulting in selective vulnerability.

The comparative DPPH assay results reveal that the antioxidant efficiency of the synthesized heterocyclic derivatives is strongly influenced by their structural features. Trolox, a chromanol-type phenolic antioxidant, displayed the lowest IC₅₀ value (14.3 µg/mL), consistent with its well-established ability to donate hydrogen

atoms and stabilize radicals through resonance. Compound (**5**) exhibited moderate activity ($IC_{50} = 338 \mu\text{g/mL}$), suggesting partial preservation of electron-donating or conjugated moieties that facilitate hydrogen-atom transfer (HAT). In contrast, compounds (**16**), (**14**), and (**11**) showed weaker activity ($IC_{50} > 1000 \mu\text{g/mL}$), implying limited radical stabilization due to steric hindrance or reduced π -delocalization. The observed trend aligns with literature reports that heterocyclic substitution can attenuate antioxidant potency when it disrupts phenolic conjugation or decreases the availability of labile hydrogen atoms.

Overall, the data support a mechanism dominated by HAT with minor contributions from single-electron transfer (SET), emphasizing that structural optimization toward enhanced conjugation and accessible hydroxyl groups could significantly improve radical-scavenging performance. As far as the antioxidant behavior is concerned, the results of the DPPH assessment test clearly indicated that the radical-scavenging activity can be related to the electronic properties of the synthesized molecules. Compounds with long conjugation and electron-giving substituents proved to have a better capacity to stabilize free radicals through resonance delocalization [63]. Heteroatoms that can transfer electron density are also present, and this further supports the mechanism of hydrogen atom transfer or single-electron transfer, which are the key processes involved in antioxidant action [64] [65].

4.6. Comparison and Contrast with the Previous Literature

Our findings are consistent with other studies that have shown antibacterial or antifungal properties of pyrazolone and pyrimidinone derivatives. Nevertheless, the current work adds its own value by correlating chemical modification to the parallel antimicrobial and antioxidant evaluation, providing a broader understanding of pyrazolone pharmacology.

4.7. Limitations

The research was limited to *in-vitro* screening and the choice of microorganisms. The cytotoxicity on mammalian cells and *in-vivo* pharmacological behavior are not known. Also, experimental validation of the actual molecular targets of antifungal effects was not conducted.

4.8. Implications

Notwithstanding these considerations, the results strongly support the pyrazolone framework as a valuable scaffold in drug-development initiatives aimed at the therapeutic benefit of antifungal and antioxidant activities.

4.9. Future Perspectives and Research Directions

Further studies should be conducted in the future to expand the biological evaluation of the synthesized compounds by carrying out additional *in vitro* and *in vivo* studies. The analysis of cytotoxicity against normal mammalian cell lines is im-

portant to determine therapeutic safety margins and indices of selectivity [66]-[68]. In addition, time-kill assays and minimum inhibitory concentration (MIC) values would provide a more detailed investigation into the potency and mode of action of antifungals [69]. It would be useful to conduct molecular docking and computational modeling studies that would aid in identifying potential biological targets and in rationalizing the observed structure-activity relationships at the molecular level. These strategies would help to guide the design of future derivatives and improve affinity for fungal enzymes or targets affecting oxidative stress.

Future research should also consider pharmacokinetic profiling (solubility, metabolic stability, and plasma protein binding) of the compound. These parameters are critical for the potential translation of promising *in vitro* activity into viable drug candidates.

Further functionalization of the pyrazolone core to create additional structural variation can also produce compounds with enhanced dual activity [70] [71]. Molecules that are more potent and selective might be obtained by the incorporation of substituents that are more likely to enhance membrane permeability or redox activity [72]. In general, the results of the current investigation offer a solid foundation for further research on pyrazolone-based heterocycles in medicinal chemistry and drug discovery studies.

5. Conclusions

A series of novel heterocyclic compounds that use a pyrazolone core was successfully synthesized and characterized in this work [73] [74]. It was possible to obtain diverse structural scaffolds, such as pyridazinone, triazinone, pyrimidinone, thiadiazinone, and oxazolone derivatives, using the synthetic strategy. The proposed pathways were substantiated by spectroscopic analyses to confirm the expected structures of the proposed reaction pathways. Biological assessments of the results indicated that the compounds did not have any observable antibacterial activity, but some of the derivatives had notable antifungal and antioxidant activity. Compounds 12 and 16 were the most effective antifungal agents against *Candida albicans*, and compounds 5, 14, and 16 showed significant radical-scavenging activity in the DPPH assay.

Collectively, these results indicate that the pyrazolone scaffold is a highly adaptable pharmacophore with the potential to produce selective antifungal and antioxidant molecules. The structural diversity generated by the simple synthetic transformations offers significant potential for further optimization and exploration of structure-activity relationships.

The results of this investigation highlight the importance of rational heterocyclic design in addressing contemporary therapeutic challenges. The synthetic efficiency of the chosen approach is demonstrated by the capacity to produce structurally distinct compounds from a common precursor. Additionally, the study's dual biological evaluation approach enhances the translational relevance of the findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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