



Conventional, Grinding and Microwave-Assisted Synthesis, Biological Evaluation of some Novel Pyrazole and Pyrazolone derivatives

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Abstract:

Under conventional, grinding and microwave methods, reaction of 3-methyl-1H-pyrazol-5-one **1** with benzene diazonium chloride, 4-carboxybenzenediazonium chloride and nitrous acid furnished the diazenyl derivatives **2**, **3** and **5**, respectively. Reaction of compound **3** with salicylaldehyde and compound **5** with malononitrile followed by hydrazine hydrate afforded compounds **4**, **6** and **7**, respectively. Compound **1** was converted to 4-bromopyrazolone **8**, which when allowed to react with urea, thiourea, o-phenylenediamine, 2-amino-3-hydroxypyridine and 2-amino-5-methylpheno furnished 4-aminopyrazolone **9-13**, respectively. While its reaction with p-phenylenediamine in 1:2 ratio gave bis-pyrazole derivative **14**. Also, the reaction of compound **1** toward thiosemicarbazide, thiourea, urea and guanidine hydrochloride gave the pyrazole derivatives **15**, **16**, **20** and **21**, respectively. The reaction of compound **16** with benzaldehyde gave Schiff base **17**, while its reaction with ethyl chloroacetate gave the thioxoimidazolidinone derivative **18**, which on reaction with thiosemicarbazide afforded imidazotriazolothione derivative **19**. Also, the reactivity of pyrazole **21** reacted with salicylaldehyde and o-chloro benzaldehyde was investigated to afford the corresponding Schiff base **22** and **23**, respectively. These entire novel scaffolds have been proofed using Elemental analysis, spectral data including IR, ¹H-NMR in addition to ¹³C-NMR, and mass spectra. These new scaffolds were screened for in vitro antimicrobial and cytotoxic activities. Most analogs revealed excellent to good results.

Keywords: Pyrazole, pyrazolone, microwave, grinding, conventional, antimicrobial, anticancer

Received; 8 June 2021, Revised form; 21 June 2021, Accepted; 21 June 2021, Available online 1 Oct. 2021

1. Introduction:

Pyrazole, pyrazolone and their fused derivatives are considered one of the most important heterocyclic compounds containing at least two nitrogen atoms which due to their biological and pharmacological activities and their application in industrial chemistry [1-3]. Pyrazole and pyrazolone are reported as antiviral [4, 5], antagonist [6], antimicrobial [7, 8], antibacterial [9, 10], anticancer [11, 12], anti-inflammatory [13, 14], analgesic [15], anthelmintic [16, 17], herbicidal [18], acaricidal and insecticidal [19], antimitotic [20], and antioxidant activities [21, 22].

It was observed that microwave irradiation and grinding methods were practically superior to conventional heating method since they showed improvement in the yield and the time of the reactions and also they were considered as green chemistry. [23, 24] On the other hand, bacteria and fungi are liable for several illnesses, most of which have public health concerns around the world.

From these findings and as continuous of our recent studies [25-31], this work aimed to investigate the behavior and reactivity of the 3-methyl-1H-pyrazol-5-one **1** [32] toward some carbon and nitrogen nucleophiles under different aspects in order to prepare novel heterocyclic compounds. On the other hand, comparison between conventional, grinding

and microwave methods by using different physical tools as YE, OE, AE, and RME. Elemental analyses together with spectroscopic data (IR, ¹H-NMR, ¹³C-NMR, and mass spectra) submit proofs for the structures for all prepared compounds. The newly synthesized compounds were tested as antibacterial against two gram-positive (*S. aureus*, *B. subtilis*) and two gram-negative bacteria, (*E. coli*, *P. aeruginosa*). The antifungal of the compounds were tested against two fungi (*C. albicans*, *A. flavus*). The newly compounds were tested also as anticancer agents against colorectal carcinoma (HCT-116) and mammary gland breast cancer (MCF-7).

2. Experimental

2.1. Synthesis:

All chemicals, starting materials, solvents and reagents were purchased from Sigma Aldrich, the solvents were dried to use by the handbook Purification of Laboratory Chemicals. Thin layer chromatography (TLC) was carried out for the monitoring of the progress of all reactions and homogeneity of the synthesized compounds, TLC was performed on pre-coated silica gel plates (Merck Kiesel gel 60F₂₅₄, BDH). All melting points were determined on a digital Stuart SMP3 electric melting point apparatus and are uncorrected.

Microwave reactor Anton Paar (monowave 300) was used for microwave irradiation 9 reactions using borosilicate glass vials of 10 mL. Infrared (IR) spectra were measured on PerkinElmer 293 spectrophotometer (cm^{-1}) using KBr disks. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were measured on Varian Mercury 300 MHz spectrometer in DMSO-d_6 as a solvent using tetramethylsilane as an internal standard. Multiplicity is denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiple) or combinations thereof. Chemical shift (δ) is measured in ppm and coupling constants (J) in Hz. The mass spectra were recorded on a GC-2010 Shimadzu Gas chromatography instrument mass spectrometer (70 eV) using the electron ionization technique. Elemental microanalyses (C, H, N) were performed on a PerkinElmer CHN-2400 analyzer and the microanalyses were found to be in good agreement within $\pm 0.4\%$ of the theoretical values.

2.1.2. Formation of 5-Methyl-4-(phenyldiazenyl)-2,4-dihydro-3H-pyrazol-3-one (2)

Aniline (0.93 mL, 0.01 mol.) was dissolved in a mixture of concentrated HCl (5 mL) and water (5 mL) and cooled to $0-5^\circ\text{C}$ in an ice bath. A cold aqueous solution of sodium nitrite (0.68g, 0.01 mol.) was added under stirring. The formed diazonium salt was filtered into a cold solution of sodium acetate (4 g) and compound **1** (0.98 g, 0.01 mol.) in ethanol (25 mL) was added under stirring for 2 h. The product was filtered off and recrystallized from ethanol (50%) to afford compound **2**.

Orange crystal; m.p. $178-180^\circ\text{C}$. IR (cm^{-1}) ν : 3143 (NH), 1662 (C=O), 1592 (C=N), 1544 (N=N). $^1\text{H-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 1.28 (s, 1H, CH), 2.11 (s, 3H, CH_3), 7.17-7.45 (m, 5H, Ar-H), 11.52 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 12.1 (CH_3), 66.4 (CH), 126.3 (CH-Ar), 127.4 (CH-Ar), 128.6 (CH-Ar), 130.2 (CH-Ar), 133.8 (CH-Ar), 149.2 (C-Ar), 155.2 (C=N), and 167.0 (C=O). MS (m/z): 202 (M^+ , 9.89%). Anal. Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}$ (202): C, 59.41; H, 4.95; N, 27.73. Found: C, 59.24; H, 5.13; N, 27.66.

2.1.3 Formation of 4-((3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)benzoic acid (3)

4-Aminobenzoic acid (1.73 g, 0.01 mol.) was dissolved in a mixture of concentrated HCl (5 mL) and water (5 mL) and cooled to $0-5^\circ\text{C}$ in an ice bath. A cold aqueous solution of sodium nitrite (0.68g, 0.01 mol.) was added under stirring. The formed diazonium salt was filtered into a cold solution of sodium acetate (4 g) and compound **1** (0.98 g, 0.01 mol.) in ethanol (25 mL) under stirring for 4 h. The product was filtered off and recrystallized from ethanol (50%) to give compound **3**.

Yellow crystal; m.p. $> 300^\circ\text{C}$. IR (cm^{-1}) ν : 3391 (OH), 3257 (NH), 1670, 1664 (C=O), 1605 (C=N), 1539 (N=N). $^1\text{H-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 1.22 (s, 1H, CH), 2.15 (s, 3H, CH_3), 7.57-7.96 (m, 4H, Ar-H), 11.61 (s, 1H, NH, D_2O exchangeable), 12.05 (s, 1H, OH, D_2O exchangeable). $^{13}\text{C-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 11.7 (CH_3), 66.9 (CH), 125.2 (CH-Ar), 127.1 (CH-Ar), 129.7 (CH-Ar), 132.8 (CH-Ar), 146.3 (C-Ar), 151.4 (C-Ar), 156.3 (C=N), 163.0 (C=O),

and 172.3 (COOH). MS (m/z): 246 (M^+ , 69.74%). Anal. Calcd for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}_3$ (246): C, 53.66; H, 4.07; N, 22.76. Found: C, 53.49; H, 4.27; N, 22.69.

2.1.4. Formation of 4-((3-(2-Hydroxystyryl)-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)benzoic acid (4)

A mixture of compound **3** (2.46 g, 0.01 mol.), salicylaldehyde (1.22 mL, 0.01 mol.), potassium hydroxide (0.56 g, 0.01 mol.) in ethanol (30 mL) was refluxed for 2h. The solid formed while heating was collected by filtration, washed with ethanol and recrystallized from methanol to produce compound **4**.

Greenish brown crystal; m.p. $> 300^\circ\text{C}$. IR (cm^{-1}) ν : 3440, 3373 (OH), 3209 (NH), 1727, 1671 (C=O), 1599 (C=N), 1574 (C=C), 1544 (N=N). $^1\text{H-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 1.18 (s, 1H, CH), 6.96-7.96 (m, 10H, Ar-H), 8.94 (s, 1H, OH, D_2O exchangeable), 11.42 (s, 1H, NH, D_2O exchangeable), 12.09 (s, 1H, COOH, D_2O exchangeable). $^{13}\text{C-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 69.3 (CH_3), 115.5 (CH-Ar), 116.7(CH-Ar), 127.5 (CH-Ar), 128.7 (CH-Ar), 130.5(CH-Ar), 130.8 (2*CH-Ar), 131.5 (CH-Ar), 133.1 (CH-Ar), 133.7 (CH-Ar), 147.1 (C-Ar), 148.0(C-Ar), 152.1 (C-Ar), 154.9 (C-Ar), 158.7 (C=N), 166.9 (C=O), and 169.3 (C=O). MS (m/z): 350 (M^+ , 27.52%). Anal. Calcd for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_4$ (350): C, 61.71; H, 4.00; N, 16.00. Found: C, 61.55; H, 3.92; N, 15.90.

2.1.5 Formation of 4-(Chlorodiazenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (5)

Compound **1** (0.98 g, 0.01 mol.) was dissolved in a mixture of HCl (5 mL) and ethanol (25 mL) and cooled to $0-5^\circ\text{C}$ in an ice bath. A cold aqueous solution of sodium nitrite (0.68g, 0.01 mol.) was added under stirring. The formed diazonium salt was filtered into a cold solution of sodium acetate (4 g) under stirring for 2 h. The product was filtered off and recrystallized from petroleum (60-80 $^\circ\text{C}$)/ethanol (1:1) to furnish compound **5**.

Yellow crystal; m.p. $240-242^\circ\text{C}$. IR (cm^{-1}) ν : 3091 (NH), 1703 (C=O), 1617 (C=N), 1591 (N=N). $^1\text{H-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 1.02 (s, 1H, CH), 2.03 (s, 3H, CH_3), 11.37 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 12.9 (CH_3), 62.5 (CH), 154.0 (C=N), and 163.7 (C=O). MS (m/z): 160 (M^+ , 17.09%). Anal. Calcd for $\text{C}_4\text{H}_5\text{ClN}_4\text{O}$ (160): C, 23.00; H, 3.13; N, 35.00; Cl, 22.19. Found: C, 29.88; H, 3.20; N, 35.13; Cl, 22.07.

2.1.6. Formation of 2-((3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)malononitrile (6)

A mixture of compound **5** (1.60 g, 0.01 mol.), malononitrile (0.66 mL, 0.01 mol.), piperidine (0.85 mL, 0.01 mol.) in ethanol (25 mL) was refluxed for 3h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from methanol to afford compound **6**.

Black powder; m.p. $160-162^\circ\text{C}$. IR (cm^{-1}) ν : 3161 (NH), 2190 (CN), 1620 (C=O), 1588 (C=N), 1559 (N=N). $^1\text{H-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 1.61 (s, 1H, CH), 2.03 (s, 3H, CH_3), 3.47 (s, 1H, $\text{CH}(\text{CN})_2$), 7.88 (s, 1H, NH, D_2O exchangeable). $^{13}\text{C-NMR}$ (300MHz, DMSO-d_6) δ (ppm): 12.7 (CH_3), 63.0 (CH), 116.8 (2*CN), 145.0 (C=N), 155.2 (C=N), and 169.2 (C=O). MS (m/z): 190 (M^+ , 15.78%). Anal.

Calcd for C₇H₆N₆O (190): C, 44.21; H, 3.16; N, 44.21. Found: C, 44.37; H, 3.22; N, 44.03.

2.1.7. Formation of 4-((3,5-Diamino-1H-pyrazol-4-yl) diazenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (7)

A mixture of compound **6** (1.90 g, 0.01 mol.), hydrazine hydrate (0.5 mL, 0.01 mol.) in butanol (20 mL) was refluxed for 6h. The solid formed after cooling was collected by filtration, washed with ethanol, and recrystallized from acetone to give compound **7**.

Brown crystal; m.p. 94-96 °C. IR (cm⁻¹) ν : 3334, 3212 (broad, NH₂ & NH), 1729 (C=O), 1626 (broad, C=N & N=N). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 1.63 (s, 3H, CH₃), 4.24 (s, 1H, CH), 6.00-7.60 (broad, 4H, 2NH₂, D₂O exchangeable), 7.68 (s, 1H, NH, D₂O exchangeable), 7.70 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.8 (CH₃), 62.1 (CH), 121.3 (C=C), 143.9 (C=N), 148.1 (C-NH₂), 149.8 (C-NH₂), and 163.5 (C=O). MS (m/z): 222 (M⁺, 35.85%). Anal. Calcd for C₇H₁₀N₈O (222): C, 37.84; H, 4.50; N, 50.45. Found: C, 37.99; H, 4.39; N, 50.42.

2.1.8. Formation of 4-Bromo-3-methyl-1H-pyrazol-5-ol (8)

To a solution of compound **1** (0.98 g, 0.01 mol.) in acetic acid (25 mL) bromine water (1.59 mL, 0.01 mol.) was added dropwise with stirring for 3h. The reaction mixture was poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from ethanol to produce compound **8**.

Off white crystal; m.p. 184-186 °C. IR (cm⁻¹) ν : 2697-3023 (OH & NH), 1620 (C=N), 1550 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.06 (s, 3H, CH₃), 4.31 (s, 1H, OH, D₂O exchangeable), 5.18 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.8 (CH₃), 89.3 (CH-Br), 144.5 (C=N), and 149.2 (C-OH). MS (m/z): 177 (M⁺, 23.28%). Anal. Calcd for C₄H₅BrN₂O (177): C, 27.12; H, 2.82; N, 15.82; Br, 45.20. Found: C, 27.30; H, 2.70; N, 15.77; Br, 45.25.

2.1.9. Formation of 1-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)urea (9)

A mixture of compound **1** (0.98 g, 0.01 mol.), urea (0.6 g, 0.01 mol.) in DMF (15 mL) was refluxed for 8h. The reaction mixture after cooling was poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from methanol to furnish compound **9**.

Brown powder; m.p. 174-176 °C. IR (cm⁻¹) ν : 2500-4000 (broad, NH₂ & NH), 1719, 1665 (C=O), 1620 (C=N). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.04 (s, 3H, CH₃), 3.49 (s, 1H, CH), 5.39 (s, 1H, NH, D₂O exchangeable), 7.12 (s, 2H, NH₂, D₂O exchangeable), 10.63 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.2 (CH₃), 63.1 (CH), 159.9 (C=N), 163.2 (C=O), and 169.2 (C=O). MS (m/z): 156 (M⁺, 3.36%). Anal. Calcd for C₅H₈N₄O₂ (156): C, 38.46; H, 5.13; N, 35.90. Found: C, 38.21; H, 5.07; N, 36.00.

2.1.10. Formation of 1-(5-Hydroxy-3-methyl-1H-pyrazol-4-yl)thiourea (10)

A mixture of compound **1** (0.98 g, 0.01 mol.), thiourea (0.76 g, 0.01 mol.) in dioxane (15 mL) was refluxed for 6h. The reaction mixture after cooling was poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from acetic to afford compound **10**.

Yellow crystal; m.p. 218-220 °C. IR (cm⁻¹) ν : 3187, 3141, 3091 (broad, OH, NH₂ & NH), 1621 (C=N), 1579 (C=C), 1246 (C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.12 (s, 3H, CH₃), 6.98 (s, 1H, NH, D₂O exchangeable), 7.11 (s, 2H, NH₂, D₂O exchangeable), 7.13 (s, 1H, OH, D₂O exchangeable), 7.24 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 10.3 (CH₃), 127.3 (C=C), 151.5 (C=N), 157.8 (C-OH), and 184.4 (C=S). MS (m/z): 172 (M⁺, 54.64%). Anal. Calcd for C₅H₈N₄OS (172): C, 34.88; H, 4.65; N, 32.56; S, 18.60. Found: C, 34.97; H, 4.81; N, 32.43; S, 18.41.

2.1.11. Formation of 4-((2-Aminophenyl)amino)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (11)

A mixture of compound **1** (0.98 g, 0.01 mol.), o-phenylenediamine (1.08 g, 0.01 mol.) in ethanol (25 mL) was refluxed for 7h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from methanol to give compound **11**.

Reddish brown crystal; m.p. 206-208 °C. IR (cm⁻¹) ν : 3357, 3308 (NH₂), 3180, 3164 (NH), 1673 (C=O), 1620 (C=N). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.06 (s, 3H, CH₃), 2.09 (s, 1H, CH), 6.67 (s, 2H, NH₂, D₂O exchangeable), 6.96 (s, 1H, NH, D₂O exchangeable), 7.60-8.05 (m, 4H, Ar-H), 12.09 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.2 (CH₃), 89.1 (CH), 123.8 (CH, Ar), 125.5 (CH, Ar), 127.3 (CH, Ar), 128.3 (CH, Ar), 138.6 (C, Ar), 140.5 (C, Ar), 157.8 (C=N), 160.4 (C=O). MS (m/z): 204 (M⁺, 24.22%). Anal. Calcd for C₁₀H₁₂N₄O (204): C, 58.82; H, 5.88; N, 27.45. Found: C, 58.94; H, 5.71; N, 27.55.

2.1.12. Formation of 2-((5-Hydroxy-3-methyl-1H-pyrazol-4-yl)amino)pyridin-3-ol (12)

A mixture of compound **1** (0.98 g, 0.01 mol.), 2-amino-3-hydroxypyridine (1.10 g, 0.01 mol.) in butanol (15 mL) was refluxed for 4h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from methanol to produce compound **12**.

Black crystal; m.p. >300 °C. IR (cm⁻¹) ν : 2400-3500 (broad, OH & NH), 1596 (C=N), 1505 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.04 (s, 3H, CH₃), 3.40 (s, 1H, CH), 6.66 (s, H, NH, D₂O exchangeable), 6.79 (s, 1H, OH, D₂O exchangeable), 7.01-7.35 (m, 3H, Ar-H), 12.24 (s, 1H, NH, D₂O exchangeable). MS (m/z): 206 (M⁺, 14.17%). Anal. Calcd for C₉H₁₀N₄O₂ (206): C, 52.43; H, 4.85; N, 27.18. Found: C, 52.29; H, 4.74; N, 27.30.

2.1.13. Formation of 4-((2-Hydroxy-4-methylphenyl)amino)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (13)

A mixture of compound **1** (0.98 g, 0.01 mol.), 2-amino-5-methylphenol (1.23 g, 0.01 mol.) in dioxane (15 mL) was refluxed for 4h. The reaction mixture after cooling was

poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from acetone to furnish compound 13.

Black powder; m.p. 210-212 °C. IR (cm⁻¹) ν : 2500-3500 (broad, OH & NH), 1662 (C=O), 1625 (C=N), 1573 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.05 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 3.70 (s, 1H, CH), 7.00 (s, 1H, NH, D₂O exchangeable), 7.17-7.44 (m, 3H, Ar-H), 7.78 (s, 1H, OH, D₂O exchangeable), 11.43 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 18.7 (CH₃), 34.8 (CH₃), 60.5 (CH), 112.5 (2*CH, Ar), 121.3 (CH, Ar), 125.2 (C, Ar), 142.4 (C, Ar), 147.6 (C, Ar), 155.3 (C=N), and 168.4 (C=O). MS (*m/z*): 219 (M⁺, 21.0%). Anal. Calcd for C₁₁H₁₃N₃O₂ (219): C, 60.27; H, 5.94; N, 19.18. Found: C, 60.40; H, 5.86; N, 19.09.

2.1.14. Formation of 4,4'-(1,4-Phenylenebis (azanediyl)) bis(5-methyl-2,4-dihydro-3H-pyrazol-3-one) (14)

A mixture of compound **1** (1.96 g, 0.02 mol.), p-phenylenediamine (1.08 g, 0.01 mol.) in acetone (25 mL) was refluxed for 7h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from acetic acid to afford compound 14.

Black powder; m.p. 140-142 °C. IR (cm⁻¹) ν : 3230, 3122 (NH), 1698, 1710 (C=O), 1572, 1560 (C=N). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.11 (s, 6H, 2CH₃), 3.36 (s, 2H, 2CH), 6.72-7.22 (m, 4H, Ar-H), 7.96 (s, 2H, 2NH, D₂O exchangeable), 10.90 (s, 2H, 2NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.3 (2*CH₃), 56.3 (2*CH), 119.2 (2*CH-Ar), 121.7 (2*CH-Ar), 144.0 (C-NH), 157.3 (2*CN), and 169.4 (2*C=O). MS (*m/z*): 300 (M⁺, 48.79%). Anal. Calcd for C₁₄H₁₆N₆O₂ (300): C, 56.00; H, 5.33; N, 28.00. Found: C, 55.87; H, 5.43; N, 28.01.

2.1.15. Formation of 2-(3-Methyl-1H-pyrazol-5-yl) hydrazine-1-carbothioamide (15)

A mixture of compound **1** (0.98 g, 0.01 mol.), thiosemicarbazide (0.91 g, 0.01 mol.), potassium hydroxide (0.56 g, 0.01 mol.) in ethanol (30 mL) was refluxed for 4h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from ethanol to give compound 15.

White crystal; m.p. 90-92 °C. IR (cm⁻¹) ν : 2955, 2918, 2849 (NH₂ & NH), 1633 (C=N), 1255 (C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.02 (s, 3H, CH₃), 6.74 (s, 1H, NH, D₂O exchangeable), 6.94 (s, 1H, Ar-H), 8.93 (s, 2H, NH₂, D₂O exchangeable), 10.20 (s, 2H, 2NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 12.3 (CH₃), 116.6 (C=C), 133.2 (C=N), 155.7 (C-NH), and 188.3 (C=S). MS (*m/z*): 171 (M⁺, 6.76%). Anal. Calcd for C₅H₉N₃S (171): C, 35.09; H, 5.26; N, 40.94; S, 18.71. Found: C, 34.92; H, 5.47; N, 40.88; S, 18.73.

2.1.16. Formation of 1-(5-Methyl-4H-pyrazol-3-yl) thiourea (16)

A mixture of compound **1** (0.98 g, 0.01 mol.), thiourea (0.76 g, 0.01 mol.), sodium hydroxide 10% (2 mL) in ethanol (30 mL) was refluxed for 5h. The solid formed after cooling

was collected by filtration, washed with water then ethanol and recrystallized from ethanol to produce compound 16.

Yellow crystal; m.p. 208-210 °C. IR (cm⁻¹) ν : 2400-3300 (broad, NH₂ & NH), 1617 (C=N), 1533 (C=C), 1250 (C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 1.97 (s, 3H, CH₃), 3.44 (s, 2H, CH₂), 7.08 (s, 3H, NH&NH₂, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.8 (CH₃), 58.2 (CH), 148.0 (C=N), 150.7 (C=N), and 181.9 (C=S). MS (*m/z*): 156 (M⁺, 2.01%). Anal. Calcd for C₅H₈N₄S (156): C, 38.46; H, 5.13; N, 35.90; S, 20.51. Found: C, 38.33; H, 5.07; N, 36.04; S, 20.56.

2.1.17. Formation of 1-Benzylidene-3-(5-methyl-4H-pyrazol-3-yl)thiourea (17)

A mixture of compound **16** (1.56 g, 0.01 mol.), benzaldehyde (1.06 mL, 0.01 mol.), sodium hydroxide (0.4 g, 0.01 mol.) in ethanol (30 mL) was stirred for 3h at room temperature. The reaction mixture was poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from petroleum (60-80) to furnish compound 17.

Reddish brown crystal; m.p. 158-160 °C. IR (cm⁻¹) ν : 3194, 3062 (NH), 1619 (C=N), 1559 (C=C), 1281 (C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 1.99 (s, 3H, CH₃), 3.39 (s, 2H, CH₂), 7.02-7.51 (m, 6H, Ar-H), 8.97 (s, 1H, NH, D₂O exchangeable). MS (*m/z*): 244 (M⁺, 34.80%). Anal. Calcd for C₁₂H₁₂N₄S (244): C, 59.02; H, 4.92; N, 22.95; S, 13.11. Found: C, 58.84; H, 5.03; N, 23.07; S, 13.06.

2.1.18. Formation of 1-(5-Methyl-4H-pyrazol-3-yl)-2-thioxoimidazolidin-4-one (18)

A mixture of compound **16** (1.56 g, 0.01 mol.), ethyl chloroacetate (1.22 mL, 0.01 mol.), potassium carbonate anhydrous (1.38 g, 0.01 mol.) in methanol (30 mL) was refluxed for 3h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from ethanol to afford compound 18.

Orange powder; m.p. 180-182 °C. IR (cm⁻¹) ν : 3200(NH), 1696 (C=O), 1623 (C=N), 1540 (C=C), 1245 (C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.03 (s, 3H, CH₃), 3.23 (s, 2H, CH₂), 4.27 (s, 2H, CH₂CO), 5.34 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 12.2 (CH₃), 52.7 (CH₂), 54.2 (CH₂), 154.7 (C=N), 156.0 (C=N), 167.2 (C=O), and 180.9 (C=S). MS (*m/z*): 196 (M⁺, 19.85%). Anal. Calcd for C₇H₈N₄OS (196): C, 42.86; H, 4.08; N, 28.57; S, 16.33. Found: C, 42.71; H, 4.24; N, 28.49; S, 16.15.

2.1.19. Formation of 3-Amino-6-(3-methyl-1H-pyrazol-5-yl)-6,7-dihydro-5H-imidazo[5,1-c][1,2,4] triazole-5-thione (19)

A mixture of compound **18** (1.96 g, 0.01 mol.), thiosemicarbazide (0.91 g, 0.01 mol.), potassium carbonate anhydrous (1.38 g, 0.01 mol.) in methanol (30 mL) was refluxed for 8h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from toluene to give compound 19.

Dark green powder; m.p. 294-296 °C. IR (cm⁻¹) ν : 3291(NH₂), 3163 (NH), 1622 (C=N), 1540 (C=C), 1276

(C=S). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.03 (s, 3H, CH₃), 3.47 (s, 2H, CH₂), 6.14 (s, 1H, Ar-H), 7.92 (s, 2H, NH₂, D₂O exchangeable), 10.43 (s, 1H, NH, D₂O exchangeable). MS (*m/z*): 235 (M⁺, 43.79%). Anal. Calcd for C₈H₉N₇S (235): C, 40.85; H, 3.83; N, 41.70; S, 13.62. Found: C, 40.76; H, 3.98; N, 41.79; S, 13.47.

2.1.20. Formation of 1-(3-Methyl-1H-pyrazol-5-yl)urea (20)

A mixture of compound **1** (0.98 g, 0.01 mol.), urea (0.6 g, 0.01 mol.) was fused for 0.5 h. The reaction mixture after cooling was poured onto cold water, the solid formed was collected by filtration, washed with water and recrystallized from ethanol to produce compound **20**.

Orang powder; m.p. > 300 °C. IR (cm⁻¹) ν: 3500-3000 (broad, NH₂ & NH), 1683 (C=O), 1610 (C=N), 1567 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 1.97 (s, 3H, CH₃), 5.47 (s, 2H, NH₂, D₂O exchangeable), 6.34 (s, 1H, Ar-H), 6.82 (s, 1H, NH, D₂O exchangeable), 8.64 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 12.3 (CH₃), 111.7 (CH-Ar), 132.5 (C-NH), 148.0 (C=N), and 170.2 (C=O). MS (*m/z*): 140 (M⁺, 24.81%). Anal. Calcd for C₅H₈N₄O (140): C, 42.86; H, 5.71; N, 40.00. Found: C, 42.77; H, 5.90; N, 39.91.

2.1.21. Formation of 1-(3-Methyl-1H-pyrazol-5-yl)guanidine (21)

A mixture of compound **1** (0.98 g, 0.01 mol.), guanidine hydrochloride (0.95 g, 0.01 mol.), potassium carbonate anhydrous (1.38 g, 0.01 mol.) in acetone (30 mL) was refluxed for 3h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from ethanol/THF (1:1) to furnish compound **21**.

Orang crystal; m.p. 180-182 °C. IR (cm⁻¹) ν: 3400-3341 (NH₂), 3155, 3090 (NH), 1651 (C=N), 1534 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 2.02 (s, 3H, CH₃), 3.44 (s, 2H, NH₂, D₂O exchangeable), 6.65 (s, 1H, Ar-H), 7.07 (s, 1H, NH, D₂O exchangeable), 8.14 (s, 1H, NH, D₂O exchangeable), 10.28 (s, 1H, NH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 11.9 (CH₃), 115.2 (CH, Ar), 140.9 (C-NH), 155.3 (C=N), and 159.6 (C=NH). MS (*m/z*): 139 (M⁺, 2.76%). Anal. Calcd for C₅H₉N₅ (139): C, 43.17; H, 6.47; N, 50.36. Found: C, 43.30; H, 6.44; N, 50.29.

2.1.22. Formation of 1-(2-Hydroxybenzylidene)-3-(5-methyl-4H-pyrazol-3-yl)guanidine (22)

A mixture of compound **21** (1.39 g, 0.01 mol.), salicylaldehyde (1.22 mL, 0.01 mol.), HCl (3 drops) in ethanol (30 mL) was refluxed for 2h. The solid formed while heating was collected by filtration, washed with ethanol and recrystallized from methanol to produce compound **22**.

Red crystal; m.p. 132-134 °C. IR (cm⁻¹) ν: 3500-3300 (broad, OH & NH), 1620, 1586 (C=N), 1530 (C=C). ¹H-NMR (300MHz, DMSO-d₆) δ (ppm): 1.87 (s, 3H, CH₃), 2.06 (s, 2H, CH₂), 5.41 (s, 1H, NH, D₂O exchangeable), 6.94-7.71 (m, 5H, Ar-H), 9.00 (s, 1H, NH, D₂O exchangeable), 11.15 (s, 1H,

OH, D₂O exchangeable). ¹³C-NMR (300MHz, DMSO-d₆) δ (ppm): 12.6 (CH₃), 116.6 (CH, Ar), 118.2 (CH, Ar), 119.6 (C, Ar), 130.7 (2*CH, Ar), 133.2 (CH, Ar), 133.4 (C, Ar), 158.7 (2*C=N), 162.6 (C-OH), and 162.7 (C=N). MS (*m/z*): 243 (M⁺, 1.82%). Anal. Calcd for C₁₂H₁₃N₅O (243): C, 59.26; H, 5.35; N, 28.81. Found: C, 59.01; H, 5.52; N, 28.66.

2.1.23. Formation of 1-(2-Chlorobenzylidene)-3-(3-methyl-1H-pyrazol-5-yl)guanidine (23)

A mixture of compound **21** (1.39 g, 0.01 mol.), o-chloro benzaldehyde (1.40 mL, 0.01 mol.), H₂SO₄ (2 drops) in butanol (30 mL) was refluxed for 3h. The solid formed after cooling was collected by filtration, washed with ethanol and recrystallized from methanol to produce compound **23**.

Brown crystal; m.p. 92-94 °C. IR (cm⁻¹) ν: 3182-3067 (NH), 1590 (C=N), 1550 (C=C). MS (*m/z*): 261 (M⁺, 29.54%). Anal. Calcd for C₁₂H₁₂N₅Cl (261): C, 55.17; H, 4.60; N, 26.82; Cl, 13.41. Found: C, 55.02; H, 4.77; N, 26.83; Cl, 13.38.

2.2. Comparison between microwave and grinding methods

In the grinding reactions, the same reactants amounts in the microwave technique. The reaction completion was illustrated by using TLC. The reaction mixtures were washed with ethanol and crystallized from the suitable solvent. The grinding and microwave reaction times were showed in (Table 1). The comparison in terms of yields and times between the prepared compounds by using grinding, microwave and conventional techniques were reported. However, we used the yield economy (YE) as a term to determine the grinding, microwave and conventional synthetic different efficiencies of the same reaction.

Calculation of YE was occurred through:

$$YE = \frac{\text{yield}\%}{\text{Reaction time "min"}}$$

In this report, the YE was used to provide the yields obtained conclusively enhanced under the grinding, microwave and conventional conditions.

The equation of RME is:

$$RME = \frac{\text{Wt of isolated product}}{\text{Wt of reactants}}$$

While OE was used for the direct comparisons between the three reaction types and can be calculated through:

$$OE = \frac{RME}{AE} \times 100.$$

So we can consider the yield economy (YE) as a metric to enhancing the conversion efficiencies of these two different synthetic methods of the same reaction. The reaction theoretical maximum efficiency were represented by using AE, while, RME gives the observed mass efficiency. The grinding and microwave reactions atomic economy (AE) have the same values due to using two different reaction conditions to obtain the same desired compounds, as shown in (Table 1).

Table (1): The comparison in terms of physical data between the synthesized compounds under grinding and microwave techniques.

| Cpd. no. | Time "min" | | | Yield % | | | YE | | | RME | | | OE | | | AE |
|----------|------------|-----|------|---------|-----|------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Th. | G. | M.W. | Th. | G. | M.W. | Th. | G. | M.W. | Th. | G. | M.W. | Th. | G. | M.W. | |
| 2 | 120 | --- | --- | 52 | --- | --- | 0.4333 | --- | --- | 30.18 | --- | --- | 42.43 | --- | --- | 71.13 |
| 3 | 240 | --- | --- | 55 | --- | --- | 0.2292 | --- | --- | 25.97 | --- | --- | 48.25 | --- | --- | 53.83 |
| 4 | 120 | 14 | 3 | 53 | 82 | 94 | 0.4417 | 5.857 | 31.33 | 39.47 | 61.06 | 70.00 | 47.81 | 73.97 | 84.80 | 82.55 |
| 5 | 120 | --- | --- | 62 | --- | --- | 0.5167 | --- | --- | 34.93 | --- | --- | 62.00 | --- | --- | 56.34 |
| 6 | 180 | 9 | 1 | 54 | 78 | 89 | 0.3000 | 8.667 | 89.00 | 28.74 | 41.51 | 47.37 | 47.05 | 67.95 | 77.54 | 61.09 |
| 7 | 360 | 10 | 1.5 | 60 | 80 | 92 | 0.1667 | 8.000 | 61.33 | 40.98 | 54.65 | 62.84 | 60.00 | 80.00 | 92.00 | 68.31 |
| 8 | 180 | --- | --- | 63 | --- | --- | 0.3500 | --- | --- | 35.18 | --- | --- | 51.08 | --- | --- | 68.87 |
| 9 | 480 | 12 | 2.5 | 60 | 83 | 94 | 0.1250 | 6.917 | 37.60 | 40.52 | 56.05 | 63.48 | 41.04 | 56.77 | 64.30 | 98.73 |
| 10 | 360 | 12 | 2 | 59 | 79 | 90 | 0.1639 | 6.583 | 45.00 | 38.73 | 51.86 | 59.08 | 39.18 | 52.46 | 59.77 | 98.85 |
| 11 | 420 | 11 | 1 | 57 | 83 | 92 | 0.1357 | 7.545 | 92.00 | 46.14 | 67.19 | 74.48 | 46.60 | 67.85 | 75.21 | 99.03 |
| 12 | 240 | 10 | 1 | 58 | 84 | 91 | 0.2417 | 8.400 | 91.00 | 40.36 | 58.46 | 63.33 | 40.75 | 59.03 | 63.94 | 99.04 |
| 13 | 240 | 10 | 1 | 63 | 83 | 93 | 0.2625 | 8.300 | 93.00 | 44.65 | 58.83 | 65.91 | 45.06 | 59.36 | 66.51 | 99.10 |
| 14 | 420 | 13 | 1.5 | 59 | 79 | 90 | 0.1405 | 6.077 | 60.00 | 48.90 | 65.47 | 74.59 | 49.55 | 66.35 | 75.59 | 98.68 |
| 15 | 240 | 9 | 2 | 60 | 81 | 91 | 0.2500 | 9.000 | 45.50 | 35.26 | 47.60 | 53.47 | 50.52 | 68.19 | 76.61 | 69.80 |
| 16 | 300 | 9 | 2.5 | 58 | 80 | 90 | 0.1933 | 8.889 | 36.00 | 34.80 | 48.00 | 54.00 | 47.74 | 65.84 | 74.07 | 72.90 |
| 17 | 180 | 10 | 2 | 62 | 84 | 89 | 0.3444 | 8.400 | 44.50 | 43.47 | 58.90 | 62.40 | 53.81 | 72.91 | 77.24 | 80.79 |
| 18 | 180 | 11 | 1.5 | 64 | 83 | 92 | 0.3556 | 7.545 | 61.33 | 27.63 | 35.53 | 37.72 | 58.64 | 75.40 | 80.05 | 47.12 |
| 19 | 480 | 13 | 2.5 | 62 | 81 | 92 | 0.1292 | 6.231 | 36.80 | 31.47 | 41.11 | 46.70 | 56.92 | 74.35 | 84.46 | 55.29 |
| 20 | 30 | 11 | 2 | 61 | 83 | 91 | 2.0333 | 7.546 | 45.50 | 54.05 | 73.54 | 80.63 | 61.00 | 83.00 | 91.00 | 88.61 |
| 21 | 180 | 10 | 2 | 60 | 82 | 89 | 0.3333 | 8.200 | 44.50 | 21.44 | 29.30 | 31.80 | 51.06 | 69.78 | 75.73 | 41.99 |
| 22 | 120 | 13 | 2.5 | 57 | 80 | 89 | 0.4750 | 6.154 | 35.60 | 40.38 | 56.68 | 63.05 | 49.35 | 69.27 | 77.06 | 81.82 |
| 23 | 180 | 14 | 2.5 | 58 | 81 | 91 | 0.3222 | 5.786 | 36.40 | 33.57 | 46.88 | 52.66 | 48.49 | 67.72 | 76.07 | 69.23 |

2.3. Antimicrobial activities:

The antimicrobial activities of the synthesized compounds were tested against a panel of two gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). The anti-fungal activity of the compounds was tested against two fungi (*Candida albicans*, *Aspergillus flavus*). Each of the compounds was dissolved in DMSO and solution of the concentration 1 mg /ml were prepared separately paper discs of Whatman filter paper were prepared with standard size (5cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. flavus*. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic Ampicillin and antifungal Colitrimazole was also recorded using the same

procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the following formula:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

At the end of the incubation period, in terms of % Activity index were recorded (Table 2) as the lowest concentration of the substance that had no visible turbidity [33, 34]. Control experiments with DMSO and uninoculated media were run parallel to the test compounds under the same conditions.

The results demonstrate that tested fungi were more sensitive to all compounds compared with bacteria. The most active compounds against fungi were 13 and 14, while the most active compounds were 8, 10, 13, 14 and 16 for Gram-negative and 10, 13, 14, 15 and 16 for Gram-positive bacteria. In addition, Gram-negative bacteria were more sensitive to the compounds compared with Gram-positive ones (Figures 1 and 2).

Table (2): Show the antimicrobial activities in terms of % Activity index for the desired derivatives.

| Cpds. No. | E. Coli | | P. Auerginosa | | S. Aureus | | B. Subtilis | | C. Albicans | | A. Flavus | |
|--------------|--|-------------------------|--|-------------------------|--|-------------------------|--|-------------------------|--|-------------------------|--|-------------------------|
| | Diamet er of inhibiti on zone (mm) | % Activi ty index | Diamet er of inhibiti on zone (mm) | % Activi ty index | Diamet er of inhibiti on zone (mm) | % Activi ty index | Diamet er of inhibiti on zone (mm) | % Activi ty index | Diamet er of inhibiti on zone (mm) | % Activi ty index | Diamet er of inhibiti on zone (mm) | % Activi ty index |
| 2 | NA | ---- | NA | ---- | NA | ---- | 2 | 8.7 | NA | ---- | 3 | 12 |
| 3 | NA | ---- | NA | ---- | NA | ---- | NA | ---- | NA | ---- | 4 | 16 |
| 4 | 5 | 20 | 8 | 34.8 | 7 | 29.2 | 6 | 26.1 | 3 | 11.1 | 10 | 40 |
| 5 | NA | ---- | NA | ---- | NA | ---- | NA | ---- | NA | ---- | 5 | 20 |
| 6 | 8 | 32 | 11 | 47.8 | 11 | 45.8 | 10 | 43.5 | 8 | 29.6 | 14 | 56 |
| 7 | 7 | 28 | 10 | 43.5 | 8 | 33.3 | 7 | 30.4 | 3 | 11.1 | 12 | 48 |
| 8 | 13 | 52 | 18 | 78.3 | 15 | 62.5 | 16 | 69.6 | 11 | 40.7 | 19 | 76 |
| 9 | 11 | 44 | 15 | 65.2 | 14 | 58.3 | 15 | 65.2 | 13 | 48.1 | 22 | 88 |
| 10 | 19 | 76 | 22 | 95.6 | 20 | 83.3 | 23 | 100 | 20 | 74.1 | 24 | 96 |
| 11 | 9 | 36 | 12 | 52.2 | 11 | 45.8 | 12 | 52.2 | 6 | 22.2 | 13 | 52 |
| 12 | NA | ---- | 2 | 8.7 | 3 | 12.5 | 3 | 13 | NA | ---- | NA | ---- |
| 13 | 15 | 60 | 20 | 86.9 | 17 | 70.8 | 19 | 82.6 | 15 | 55.5 | 22 | 88 |
| 14 | 15 | 60 | 21 | 91.3 | 18 | 75 | 20 | 86.9 | 25 | 92.6 | 24 | 96 |
| 15 | 12 | 48 | 16 | 69.6 | 15 | 62.5 | 17 | 73.9 | 18 | 66.7 | 23 | 92 |
| 16 | 16 | 64 | 21 | 91.3 | 17 | 70.8 | 18 | 78.3 | 12 | 44.4 | 20 | 80 |
| 17 | 3 | 12 | 5 | 21.7 | 6 | 25 | 5 | 21.7 | NA | ---- | 6 | 24 |
| 18 | NA | ---- | NA | ---- | NA | ---- | NA | ---- | NA | ---- | NA | ---- |
| 19 | 8 | 32 | 11 | 47.8 | 10 | 41.7 | 10 | 43.5 | NA | ---- | 8 | 32 |
| 20 | 10 | 40 | 13 | 56.5 | 12 | 50 | 13 | 56.5 | 9 | 33.3 | 15 | 60 |
| 21 | 10 | 40 | 13 | 56.5 | 12 | 50 | 13 | 56.5 | 10 | 37 | 18 | 72 |
| 22 | 6 | 24 | 9 | 39.1 | 9 | 37.5 | 8 | 34.8 | 5 | 18.5 | 13 | 52 |
| 23 | NA | ---- | 4 | 17.4 | 5 | 20.8 | 3 | 13 | NA | ---- | 6 | 24 |
| Ampicillin | 25 | 100 | 23 | 100 | 24 | 100 | 23 | 100 | NA | ---- | NA | ---- |
| Colitrimaz | NA | ---- | NA | ---- | NA | ---- | NA | ---- | 27 | 100 | 25 | 100 |

NA → No Activity.

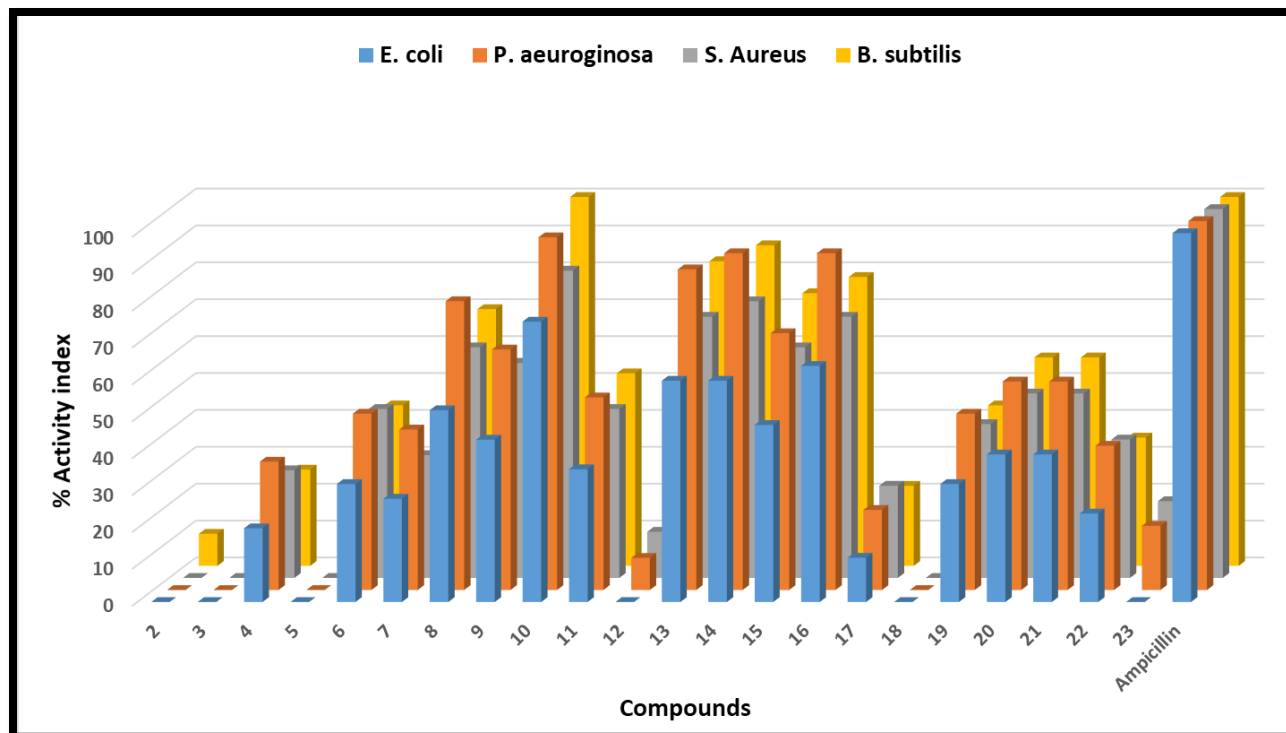


Fig (1): Percent activity of most potent compounds against bacteria.

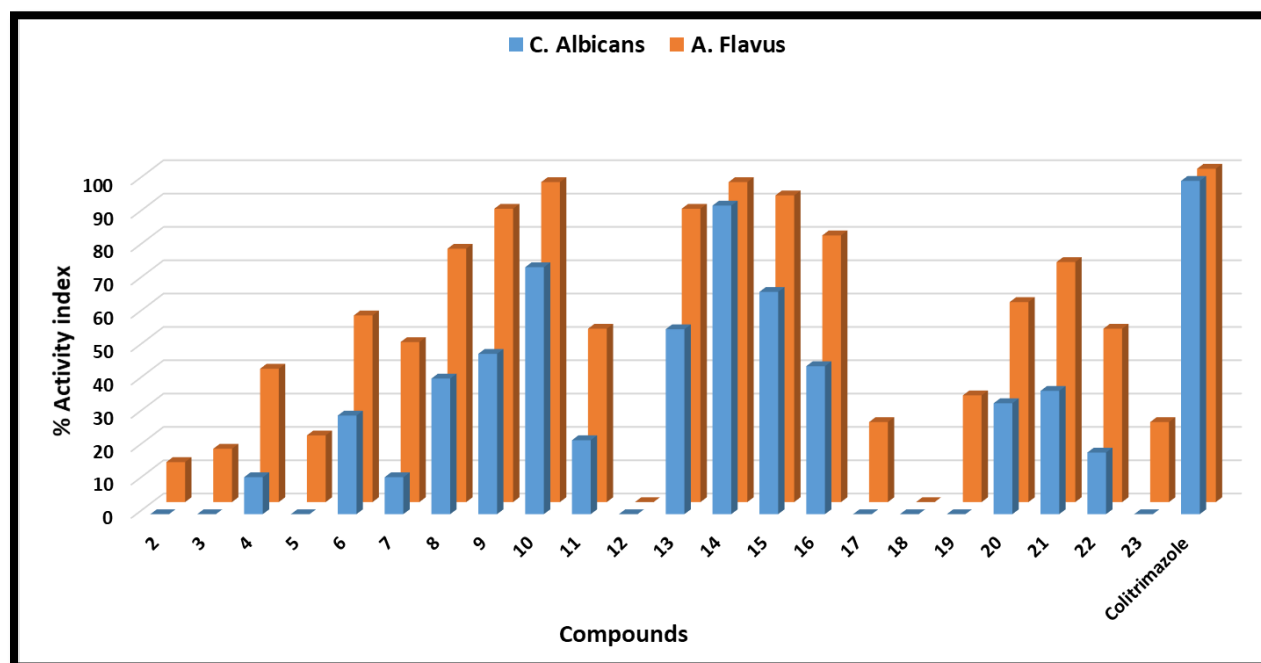


Fig (2): Percent activity index of most potent compounds against fungal.

2.4. Cytotoxicity assay

Cell line: colorectal carcinoma (HCT-116) and mammary gland breast cancer (MCF-7). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Chemical reagents: The

reagents RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK). Doxorubicin was used as a standard anticancer drug for comparison. MTT assay (1): The different cell lines

mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 °C in a 5% CO₂ incubator. The cells were seeded in a 96-well plate at a density of 1.0x10⁴ cells/well at 37 °C for 48 h under 5%

CO₂. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded (Table 3) at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A₅₇₀ of treated samples/A₅₇₀ of untreated sample) X 100. [35]

Table (3): Anticancer Activity as mean zones of inhibition (mm) against some microorganisms.

| Compounds | In vitro Cytotoxicity IC ₅₀ (µM)• | |
|-----------|--|-----------|
| | HCT-116 | MCF-7 |
| DOX | 5.23±0.3 | 4.17±0.2 |
| 2 | 91.25±4.7 | 77.34±4.1 |
| 3 | 84.36±4.3 | >100 |
| 4 | 58.35±3.3 | 63.97±3.2 |
| 5 | 78.06±4.1 | >100 |
| 6 | 42.43±2.7 | 45.27±2.5 |
| 7 | 52.40±3.1 | 54.53±2.8 |
| 8 | 28.08±2.1 | 18.12±1.4 |
| 9 | 19.10±1.4 | 26.39±1.8 |
| 10 | 7.58±0.4 | 6.28±0.3 |
| 11 | 47.18±2.8 | 39.03±2.3 |
| 12 | >100 | 71.58±3.7 |
| 13 | 11.85±0.9 | 14.76±1.1 |
| 14 | 4.63±0.2 | 12.51±0.9 |
| 15 | 8.14±0.6 | 22.35±1.6 |
| 16 | 23.72±1.8 | 9.48±0.7 |
| 17 | 70.33±3.7 | 65.71±3.4 |
| 18 | >100 | 89.39±4.4 |
| 19 | 66.24±3.5 | 48.53±2.6 |
| 20 | 36.39±2.4 | 34.16±2.1 |
| 21 | 33.67±2.3 | 31.78±2.0 |
| 22 | 49.57±2.9 | 57.89±3.0 |
| 23 | 73.94±3.8 | 68.01±3.5 |

• IC₅₀ (µM): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and >100 (non-cytotoxic)

••DOX: Doxorubicin.

The results in Table 3 revealed that compounds 10 and 16 showed a very strong cytotoxic activity and compounds 8, 9 and 14 showed a strong cytotoxic activity against MCF-7. While, revealed that compounds 10, 14 and 15 showed a very strong cytotoxic activity and compounds 9 and 13 showed a strong cytotoxic activity against HCT-116.

3. Results and discussion:

Synthesis:

The new pyrazole and pyrazolone derivatives were prepared following the reaction sequences depicted in Scheme 1-4. The starting pyrazolone **1** was prepared by a

literature known procedure [32] using ethyl acetoacetate and hydrazine hydrate in a one-pot reaction.

The interesting pharmacological properties of diazenyl derivatives as antibacterial [36], antifungal [37], anticancer [38], anti-inflammatory [39] and antioxidant [40] led us to synthesize new diazenyl derivatives **2** and **3**, respectively.

Therefore, the reaction of compound **1** with benzene diazonium chloride under stirring for 2h at 0-5°C in aqueous medium afforded phenyl diazenyl derivative **2** through electrophilic substitution reaction of the diazonium group on the carbon of CH₂ group of pyrazolone followed by

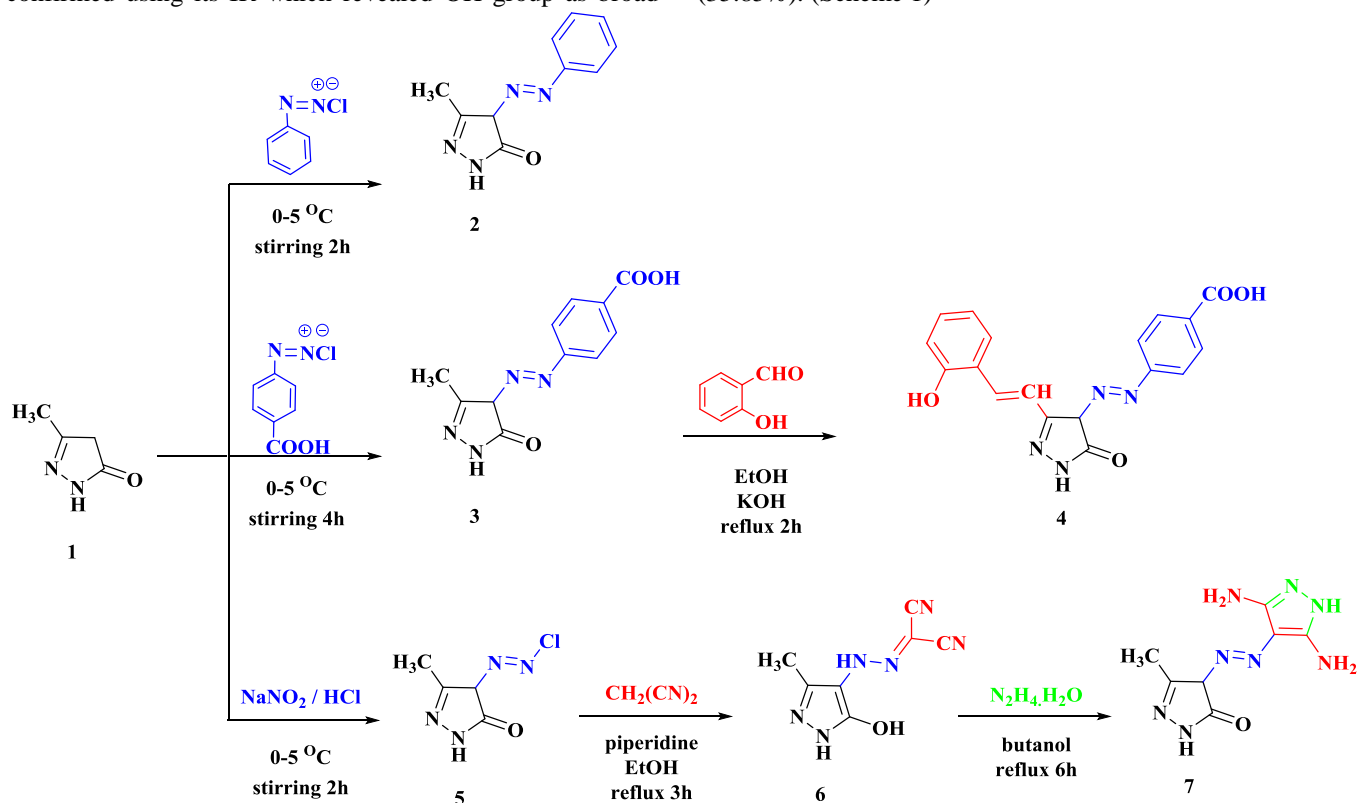
elimination one molecule of HCl. The structure of compound 2 was illustrated using its IR which displayed N=N group at ν 1544 cm^{-1} . Its $^1\text{H-NMR}$ revealed the five aromatic protons at δ 7.17–7.45 ppm. Further support for the assigned structure 2 was acquired from its mass spectrum which disclosed the correct molecular ion peak at m/z 202 (9.89%).

Similarly, compound 1 reacted with 4-carboxybenzene diazonium chloride producing diazenyl derivative 3. The IR spectrum displayed acid C=O at ν 1670 cm^{-1} , pyrazolone C=O at ν 1664 cm^{-1} and N=N group at ν 1539 cm^{-1} . Its $^1\text{H-NMR}$ showed the four aromatic protons at δ 7.57–7.96 ppm. Its $^{13}\text{C-NMR}$ showed peaks of C=O and COO at δ 163.0 and 172.3 ppm, respectively. Its mass spectrum revealed the molecular ion peak at m/z 246 (69.74%).

Further support for the assigned structure 3 was acquired from its reaction with salicylaldehyde under refluxing in ethanol in the presence of potassium hydroxide to produce the pyrazolone derivative 4. The structure of compound 2 was confirmed using its IR which revealed OH group as broad

band at ν 3440, 3373 cm^{-1} . Its $^1\text{H-NMR}$ disclosed OH group at δ 12.09 ppm and its mass spectrum which showed the molecular ion peak at m/z 350 (27.52%).

It has been found that pyrazolone derivatives 1 reacted with nitrous acid at 0–5 $^{\circ}\text{C}$ in situ prepared from the reaction of (NaNO_2 and HCl) to give the corresponding 4-chlorodiazenyl derivative 5. IR spectrum of 5 revealed the azo group at ν 1591 cm^{-1} . Its mass spectrum which showed the molecular ion peak at m/z 160 (17.09%). Azo coupling of the pyrazolo diazonium chloride 5 with malononitrile was achieved by refluxing in ethanol and few drops of piperidine to furnish the corresponding compound 6. The structure formula of compound 6 was secured by its analytical and spectral data. Its IR spectrum of compound 6 showed CN group at ν 2190 cm^{-1} . Structure of compound 6 was further supported by its reaction with hydrazine hydrate in boiling butanol to achieve the diazo pyrazole derivative 7. Mass spectrum of compound 7 which disclosed the molecular ion peak at m/z 222 (35.85%). (Scheme 1)



Scheme 1. Synthesis of diazopyrazolone derivatives 1-7.

Noteworthy, the 4-bromopyrazole derivative 8 was obtained upon bromination of compound 1 with bromine water in glacial acetic acid. The $^1\text{H-NMR}$ spectrum of 8 revealed an exchangeable singlet signal for NH_2 and exchangeable singlet for OH.

Furthermore, the structure of compound 8 was supported by its reaction with urea and thiourea to give pyrazolone derivatives 9 and 10, respectively. IR spectrum of compound

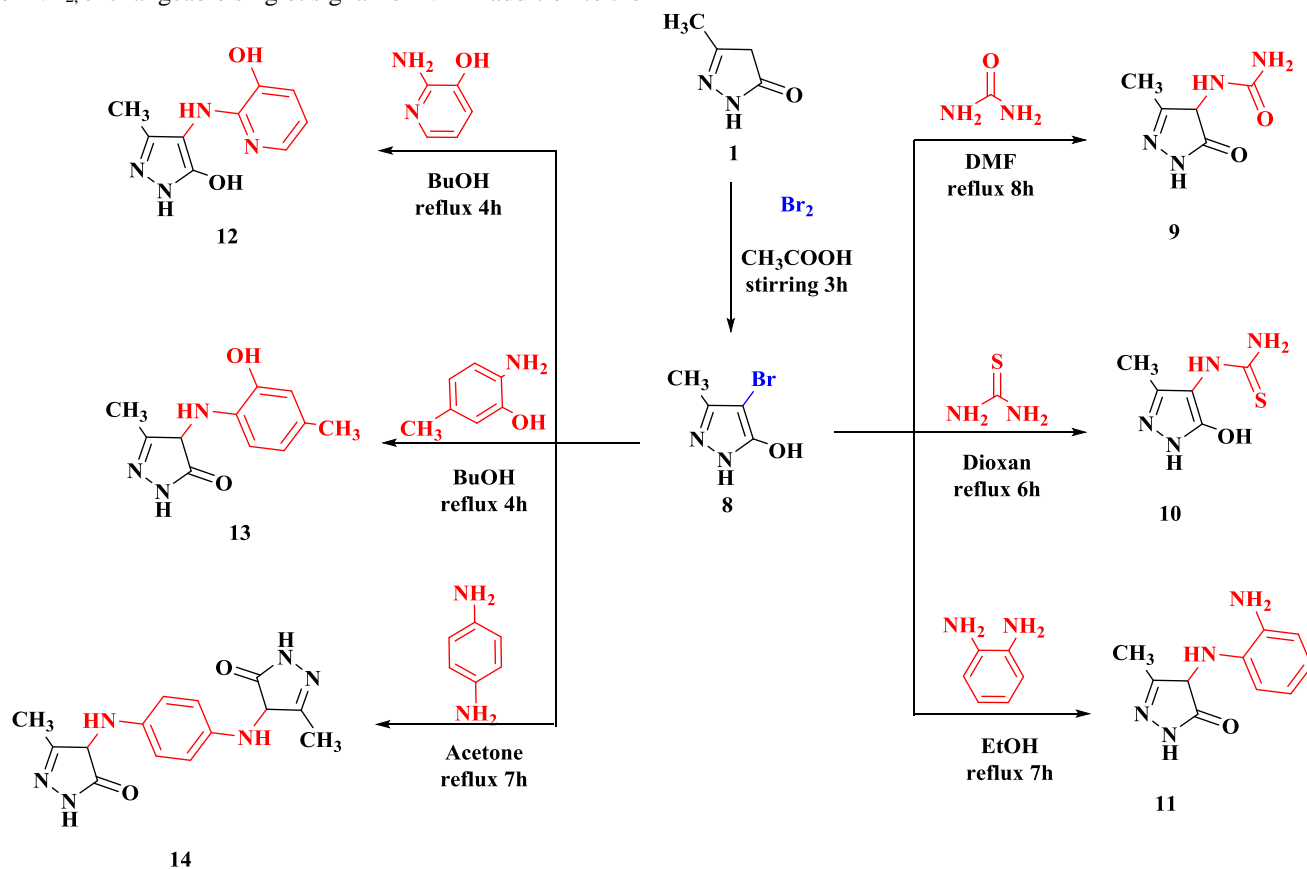
9 disclosed bands characteristic for NH_2 , NH, $2^{\circ}\text{C}=\text{O}$, and C=N groups. Its $^{13}\text{C-NMR}$ spectrum revealed peaks of C=O at δ 163.2 and 169.2 ppm. Its mass spectrum showed the molecular ion peak at m/z 156 (3.36%). While, IR spectrum of compound 10 disclosed bands characteristic for OH, NH_2 , NH, C=S, and C=N groups. Its $^{13}\text{C-NMR}$ spectrum revealed peaks of C=O and C=S at δ 157.8 and 184.4 ppm. Its mass spectrum which showed the molecular ion peak at m/z 172

(54.64%). It is worth to mention that attempt to synthesize the annulated compound by condensation of the amino group with the carbonyl group in the pyrazolone ring in compound 9 by repeating the reaction in boiling butanol for 20h has failed and the open structure 9 was obtained. The obtained products are identical in all spectra (IR spectra, m.p. and mixed m.p. as well as TLC).

On the other hand, compound 8 when allowed to react with *o*-phenylenediamine, 2-amino-3-hydroxypyridine and 2-amino-5-methylpheno the pyrazolone derivatives 11-13 were obtained, respectively. IR spectrum of compound 11 showed bands characteristic for NH₂, NH, C=O, and C=N groups. Its ¹H-NMR spectrum displayed an exchangeable singlet signal for NH₂, exchangeable singlet signal for NH in addition to the

aromatic protons. Its mass spectrum which revealed the molecular ion peak at *m/z* 204 (24.22%). IR spectrum of compound 12 disclosed bands characteristic for OH, NH, C=O, and C=N groups. Its ¹H-NMR spectrum showed an exchangeable singlet signal for OH, exchangeable singlet signal for NH in addition to the aromatic protons. Its mass spectrum which revealed the molecular ion peak at *m/z* 206 (14.17%). ¹³C-NMR spectrum of compound 13 displayed peaks of 2*CH₃ at δ 18.7 and 34.8 ppm.

Similarly, bis-pyrazolone 14 was obtained via reaction of compound 8 with *p*-phenylenediamine in a ratio 1:2. Mass spectrum of compound 14 which revealed the molecular ion peak at *m/z* 300 (48.79%). (Scheme 2)



Scheme 2. Synthesis of pyrazolone derivatives 8-14.

Interaction of pyrazolone 1 with thiosemicarbazide in boiling ethanol to furnish the pyrazole derivative 15. Its IR and ¹³C-NMR spectra were devoid of C=O band and appearance of C=S band. Further, the highest recorded peak in its mass spectrum was at *m/z* 171 (6.76%) corresponding to the correct molecular ion peak.

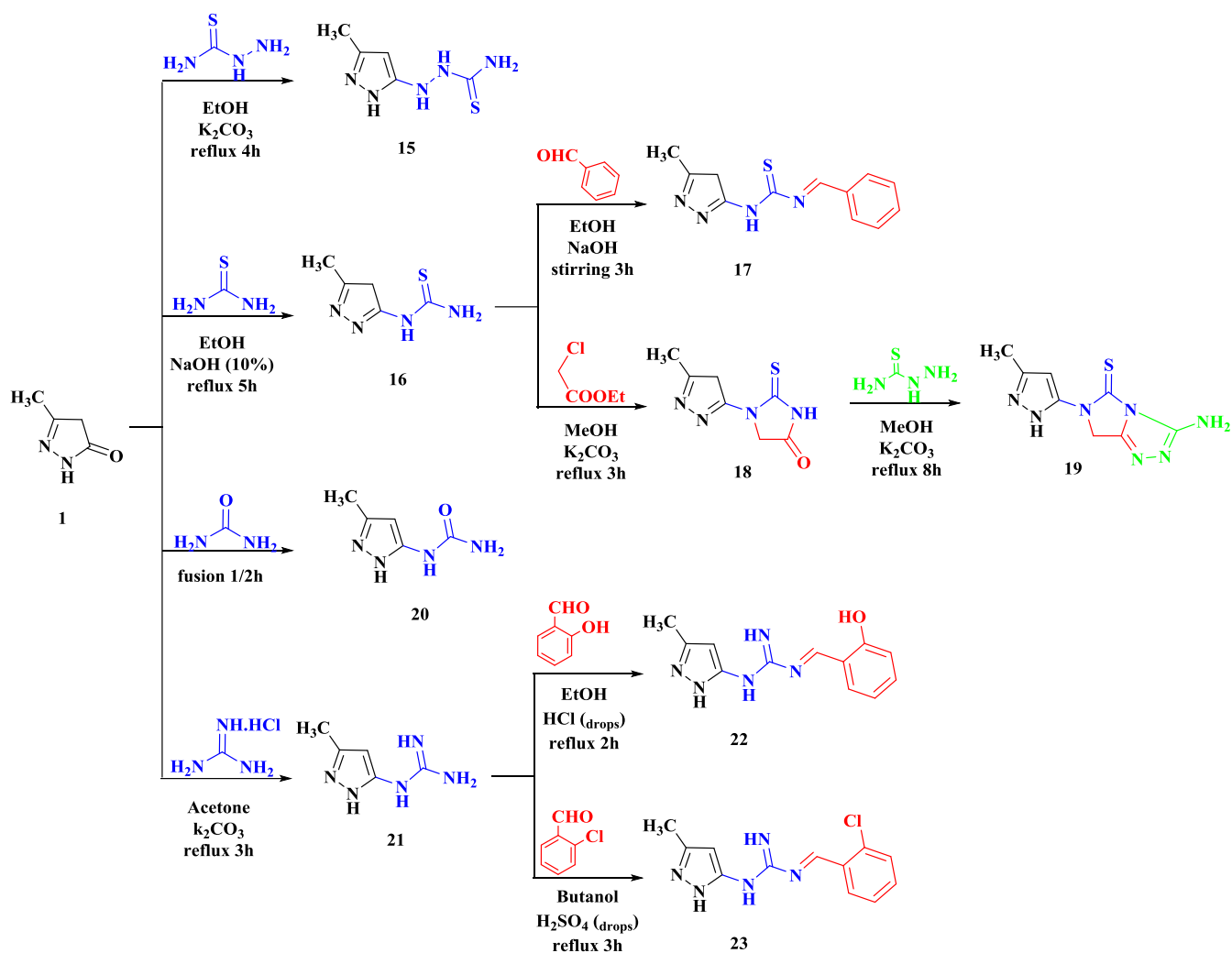
Similarly, pyrazole derivative 16 was produced from the reaction of compound 1 with thiourea in boiling ethanol in presence of NaOH. Its ¹³C-NMR spectra displayed band of C=S at δ 181.9 ppm. Its mass spectrum disclosed molecular ion peak at *m/z* 156 (2.01%). Furthermore, the structure of

compound 16 was supported by its reaction with benzaldehyde to afford the Schiff base derivative 17. Its IR spectrum was devoid of NH₂ band. Its ¹H-NMR showed the five aromatic protons at δ 7.02-7.51 ppm. Its mass spectrum which revealed the molecular ion peak at *m/z* 244 (34.80%). (Scheme 3)

While thioxoimidazolidinone derivative 18 was obtained from the reaction of compound 16 with ethyl chloroacetate, via nucleophilic attack of NH₂ group of pyrazole on C=O group of acetate followed by elimination one molecule of ethanol, then ring closure through nucleophilic attack of NH

group on CH_2Cl followed by elimination one molecule of HCl producing compound 18. The structure of compound 18 was conformed from its IR spectrum which display band of $\text{C}=\text{O}$

at ν 1696 cm^{-1} . Its ^{13}C -NMR spectra displayed band of $\text{C}=\text{O}$ at δ 167.2 ppm. Its mass spectrum disclosed molecular ion peak at m/z 196 (19.85%).

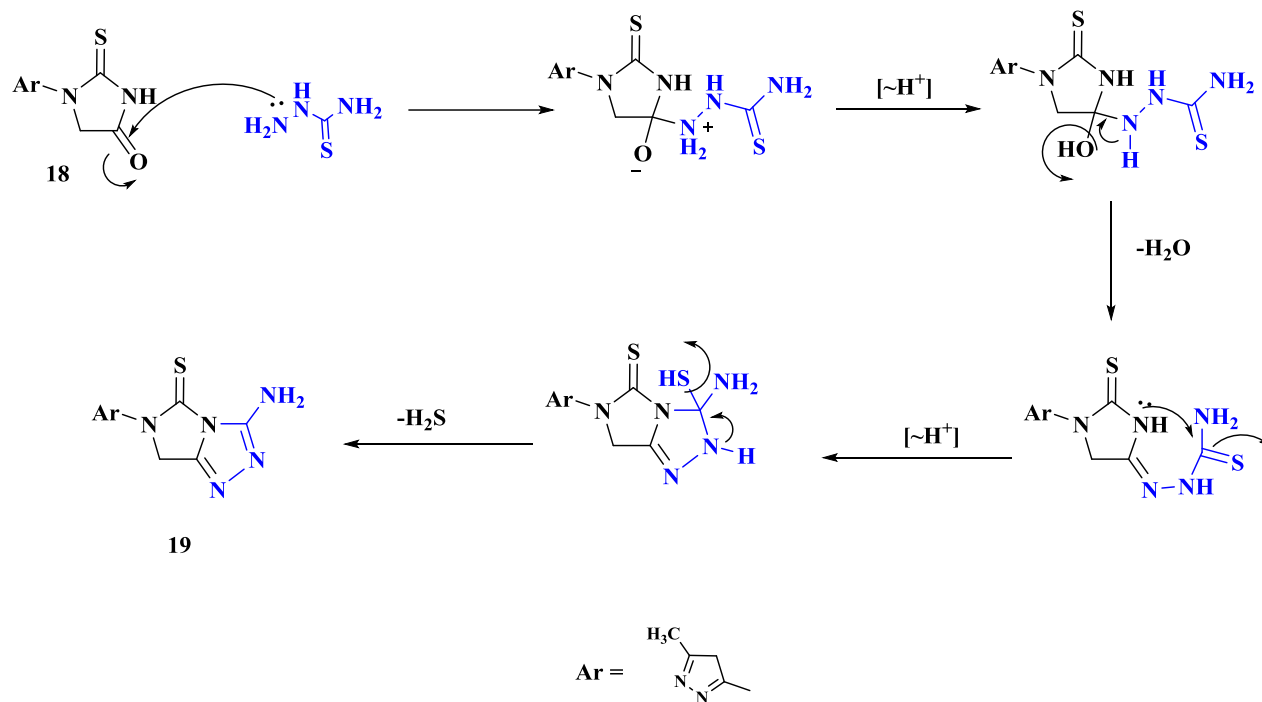


Scheme 3. synthesis of pyrazole derivatives 15-23.

Also, the structure of compound 18 was supported by its reaction with thiosemicarbazide to afford the imidazotriazolothione derivative 19. A plausible mechanism for the formation of compound 19 is shown in Scheme 4. Its IR and ^{13}C -NMR spectra were devoid of $\text{C}=\text{O}$ band. Its ^1H -NMR showed an exchangeable singlet signal for NH_2 at δ 7.92 ppm. Its mass spectrum disclosed molecular ion peak at m/z 235 (34.79%).

However, reaction of pyrazolone 1 with urea and guanidine hydrochloride gave pyrazole derivatives 20 and 21, respectively. The structure of compound 20 was conformed

from its IR spectrum which display band of $\text{C}=\text{O}$ at ν 1683 cm^{-1} . Its ^1H -NMR showed the bands of NH_2 at δ 5.47 ppm. Its ^{13}C -NMR spectra displayed band of $\text{C}=\text{O}$ at δ 170.2 ppm. The structure of compound 21 was illustrated from its mass spectrum disclosed molecular ion peak at m/z 139 (2.76%). On the other hand, the structure of compound 21 was supported via formation of Schiff base derivatives 22 and 23 by its reaction with salicylaldehyde and *o*-chloro benzaldehyde, respectively. Their IR and ^1H -NMR spectrum were devoid of NH_2 band. (Scheme 3)



Scheme 4. The mechanism of formation compound 19.

4. Conclusion:

The target of the presence search was to study the behavior and reactivity of the 3-methyl-1H-pyrazol-5-one **1** toward some nucleophilic and electrophilic reagents under conventional, grinding and microwave methods. The newly

synthesized compounds was discovered as antimicrobial and anticancer agents. The data showed clearly that some of these compounds displayed very strong; strong to moderate in in vitro antimicrobial and anticancer activities.

Acknowledgement

The authors thank Mr. Ahmed Abbas, a researcher at the Drugs Department, Faculty of Pharmacy, Mansoura University, Egypt, for kindly providing technical assistance during the antitumor activity testing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethic approval and consent to participate

Not applicable.

Human and Animal rights

No animals/humans were used for studies that are the base of this research.

Consent for publication

Not applicable

Funding statement

No funding.

References:

[1] G. H. Sayed, M. E. Azab, K. E. Anwer, M. A. Raouf, N. A. Negm, "Pyrazole, pyrazolone and enamionitrile pyrazole derivatives: Synthesis, characterization and potential in corrosion inhibition and antimicrobial applications", *Journal of Molecular Liquids*, Vol. 252, pp. 329-338, 2018.

[2] X. Gao, Q. Huang, D. Ma, Y. Jiang, T. Ren, X. Guo, L. Guo, "Improving environmental adaptability and long-term corrosion resistance of Mg alloys by pyrazole ionic liquids: Experimental and theoretical studies", *Journal of Molecular Liquids*, Vol. 333, pp. 115964-115974, 2021.

- [3] S. Hadisaputra, A. A. Purwoko, S. Hamdiani, "Substituents effects on the corrosion inhibition performance of pyrazolone against carbon steels: quantum chemical and Monte Carlo simulation studies", *Int. J. Corros. Scale Inhib*, Vol. 10, pp. 419-440, 2021.
- [4] J. Achuthanandhan, B. Lakshmanan, "Docking studies of tetra substituted pyrazolone derivatives as potential antiviral agents", *Journal of Pharmaceutical Chemistry*, Vol. 5, pp. 5-8, 2018.
- [5] G. Yang, H. Zheng, W. Shao, L. Liu, Z. Wu, "Study of the in vivo antiviral activity against TMV treated with novel 1-(t-butyl)-5-amino-4-pyrazole derivatives containing a 1, 3, 4-oxadiazole sulfide moiety", *Pesticide Biochemistry and Physiology*, Vol. 171, pp. 104740-104750, 2021.
- [6] C. A. Dvorak, J. Liang, N. S. Mani, N. I. Carruthers, "Regioselective assembly of fused pyrazole-azepine heterocycles: Synthesis of the 5-HT7 antagonist 1-benzyl-3-(4-chlorophenyl)-1, 4, 5, 6, 7, 8-hexahydropyrazolo [3, 4-d] azepine", *Tetrahedron Letters*, Vol. 67, pp. 152843-152852, 2021.
- [7] G. H.Sayed, N. A. Negm, M. E. Azab, K. E. Anwer, "Synthesis, Characterization and Biological Activity of Some Pyrazole-Pyrazolone Derivatives", *Egyptian Journal of Chemistry*, Vol. 59, pp. 663-672, 2016.
- [8] S. S. Mohamed, S. N. Shabaan, N. F. Abdelghaffar, N. T. Dauoud, G. H. Sayed, K. E. Anwer, "Synthesis and Exploring Novel Annulated 1, 3-diphenylpyrazole Derivatives as Antimicrobial and Anticancer Agents", *Journal of Basic and Environmental Sciences*, Vol. 8, pp. 124-139, 2021.
- [9] N. C. Desai, D. V. Vaja, S. B. Joshi, V. M. Khedkar, "Synthesis and molecular docking study of pyrazole clubbed oxazole as antibacterial agents", *Research on Chemical Intermediates*, Vol. 47, PP. 573-587, 2021.
- [10] I. Sehout, H. Boulebd, R. Boulcina, S. Nemouchi, L. Bendjeddou, A. Bramki, A. Debache, "Synthesis, crystal structure, Hirshfeld surface analysis, biological evaluation, DFT calculations, and in silico ADME analysis of 4-arylidene pyrazolone derivatives as promising antibacterial agents", *Journal of Molecular Structure*, Vol. 1229, pp. 129586, 2021.
- [11] R. Kshatriya, P. Shelke, S. Mali, G. Yashwantrao, A. Pratap, S. Saha, "Synthesis and Evaluation of Anticancer Activity of Pyrazolone Appended Triarylmethanes (TRAMs)" *ChemistrySelect*, Vol. 6, pp. 6230-6239, 2021.
- [12] I. M. Othman, Z. M Alamshany, N. Y. Tashkandi, M. A. Gad-Elkareem, M. M. Anwar, E. S. Nossier, "New pyrimidine and pyrazole-based compounds as potential EGFR inhibitors: synthesis, anticancer, antimicrobial evaluation and computational studies", *Bioorganic Chemistry*, Vol. 114, pp. 105078, 2021.
- [13] S. S. Abd El-Karim, H. S. Mohamed, M. F. Abdelhameed, A. E. G. E. Amr, A. A. Almezizia, E. S. Nossier, "Design, synthesis and molecular docking of new pyrazole-thiazolidinones as potent anti-inflammatory and analgesic agents with TNF- α inhibitory activity", *Bioorganic Chemistry*, Vol. 111, pp. 104827, 2021.
- [14] M. A. Shabaan, A. M. Kamal, S. I. Faggal, A. E. Elsahar, K. O. Mohamed, "Synthesis and biological evaluation of pyrazolone analogues as potential anti-inflammatory agents targeting cyclooxygenases and 5-lipoxygenase", *Archiv der Pharmazie*, Vol. 353, pp. 1900308, 2020.
- [15] R. Kenchappa, Y. D. Bodke, "Synthesis, analgesic and anti-inflammatory activity of benzofuran pyrazole heterocycles", *Chemical Data Collections*, Vol. 28, pp. 100453, 2020.
- [16] Y. Jiao, S. Preston, H. Song, A. Jabbar, Y. Liu, J. Baell, R. B. Gasser, "Assessing the anthelmintic activity of pyrazole-5-carboxamide derivatives against *Haemonchus contortus*", *Parasites & vectors*, Vol. 10, pp. 1-7, 2017.
- [17] S. M. Bairagi, R. S. Mantri, N. Nema, "evaluation of anthelmintic activity of momordica charantia fruit extract", *Vol. 7, pp. 57-60, 2011.*
- [18] S. Sangwan, R. Singh, S. Gulati, S. Rana, "Efficient and facile synthesis of pyrazoles using Guar-gum as organocatalyst and their in vitro herbicidal activity", *Current Research in Green and Sustainable Chemistry*, Vol. 4, pp. 100146, 2021.
- [19] S. Chen, Y. Zhang, Y. Liu, Q. Wang, "Highly Efficient Synthesis and Acaricidal and Insecticidal Activities of Novel Oxazolines with N-Heterocyclic Substituents", *Journal of Agricultural and Food Chemistry*, Vol. 69, pp. 3601-3606, 2021.
- [20] P. Novais, P. Silva, I. Amorim, H. Bousbaa, "Second-Generation Antimitotics in Cancer Clinical Trials. *Pharmaceutics*", Vol. 13, pp. 1011, 2021.
- [21] E. ÇINAR, E. BAŞARAN, Ö. ERDOĞAN, R. ÇAKMAK, B. O. Ğ. A. Mehmet, Ö. ÇEVİK, "Synthesis and biological evaluation of some pyrazolone based Schiff base derivatives as enzymes inhibitors, antioxidant, and anticancer agents", pp. 1-22, 2021.
- [22] S. Naveen, K. Kumara, A. D. Kumar, K. A. Kumar, A. Zarrouk, I. Warad, N. K. Lokanath, "Synthesis, characterization, crystal structure, Hirshfeld surface analysis, antioxidant properties and DFT calculations of a novel pyrazole derivative: Ethyl 1-(2,4-dimethylphenyl)-3-methyl-5-phenyl-1H-pyrazole-4-carboxylate", *Journal of Molecular Structure*, Vol. 1226, pp. 129350, 2021.
- [23] A. Dandia, S. L. Gupta, R. Sharma, P. Saini, V. Parewa, "Microwave-assisted catalyst-free organic synthesis", In *Green Sustainable Process for Chemical and Environmental Engineering and Science*, pp. 539-622, 2021.
- [24] P. Ying, J. Yu, W. Su, "Liquid-Assisted Grinding Mechanochemistry in the Synthesis of Pharmaceuticals", *Advanced Synthesis & Catalysis*, Vol. 363, pp. 1246-1271, 2021.
- [25] S. Mohamed, N. Dawoud, S. N. Shabaan, N. Fathall, G. Hosni, K. E. Anwer, "Synthesis and biological activity

of a new class of enamionitrile pyrazole”, *Egyptian Journal of Chemistry*, Vol. 64, pp. 9-10, 2021.

[26] G. H. Sayed, M. E. Azab, N. A. Negm, K. E. Anwer, “Antimicrobial and cytotoxic activities of some novel heterocycles bearing pyrazole moiety”, *Journal of Heterocyclic Chemistry*, Vol. 55, pp. 1615-1625, 2018.

[27] K. E. Anwer, A. A. Farag, E. A. Mohamed, E. M. Azmy, G. H. Sayed, “Corrosion inhibition performance and computational studies of pyridine and pyran derivatives for API X-65 steel in 6 M H₂SO₄”, *Journal of Industrial and Engineering Chemistry*, Vol. 97, pp. 523-538, 2021.

[28] A. A. Farag, E. A. Mohamed, G. H. Sayed, K. E. Anwer, “Experimental/computational assessments of API steel in 6 M H₂SO₄ medium containing novel pyridine derivatives as corrosion inhibitors”, *Journal of Molecular Liquids*, Vol. 330, pp. 115705, 2021.

[29] G. H. Sayed, M. E. Azab, K. E. Anwer, “Conventional and Microwave-Assisted Synthesis and Biological Activity Study of Novel Heterocycles Containing Pyran Moiety”, *Journal of Heterocyclic Chemistry*, Vol. 56, pp. 2121-2133, 2019.

[30] K. Anwer, G. H. Sayed, H. Hassan, M. Azab, “Conventional and Microwave Synthesis of Some New Pyridine Derivatives and Evaluation Their Antimicrobial and Cytotoxic Activities”, *Egyptian Journal of Chemistry*, Vol. 62, pp. 707-726, 2019.

[31] K. E. Anwer, G. H. Sayed, “Conventional and microwave reactions of 1, 3-diaryl-5, 4-enamionitrile-pyrazole derivative with expected antimicrobial and anticancer activities”, *Journal of Heterocyclic Chemistry*, Vol. 57, pp. 2339-2353, 2020.

[32] R. Prajuli, J. anerjee, H. E. M. A. N. T. A. Khanal, “Synthesis of some pyrazolone derivatives and evaluation of its antibacterial and cytotoxic activity” *Oriental Journal of Chemistry*, Vol. 31, pp. 2099-2106, 2015.

[33] I. Stylianakis, A. Kolocouris, N. Kolocouris, G. Fytas, G. B. Foscolos, E. Padalko, E. De Clercq, “Spiro [pyrrolidine-2, 2'-adamantanes]: synthesis, anti-influenza virus activity and conformational properties”, *Bioorganic & medicinal chemistry letters*, Vol. 13, pp. 1699-1703, 2003.

[34] R. R. Zaky, T. A. Yousef, K. M. Ibrahim, “Co (II), Cd (II), Hg (II) and U (VI) O₂ complexes of o-hydroxyacetophenone [N-(3-hydroxy-2-naphthoyl)] hydrazone: Physicochemical study, thermal studies and antimicrobial activity”, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 97, pp. 683-694, 2012.

[35] F. Denizot, R. Lang, “Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability”, *Journal of immunological methods*, Vol. 89, pp. 271-277, 1986.

[36] L. Gao, H. Zhang, B. Yu, W. Li, F. Gao, K. Zhang, H. Cong, “Chitosan composite hydrogels cross-linked by multifunctional diazo resin as antibacterial dressings for improved wound healing”, *Journal of Biomedical Materials Research Part A*, Vol. 108, pp. 1890-1898, 2020.

[37] M. N. Khan, D. K. Parmar, D. Das, “Recent Applications of Azo Dyes: A Paradigm Shift from Medicinal Chemistry to Biomedical Sciences”, *Mini Reviews in Medicinal Chemistry*, Vol. 21, pp. 1071-1084, 2021.

[38] J. D. Tadić, J. M. Ladarević, Ž. J. Vitnik, V. D. Vitnik, T. P. Stanojković, I. Z. Matić, D. Ž. Mijin, “Novel azo pyridone dyes based on dihydropyrimidinone skeleton: Synthesis, DFT study and anticancer activity”, *Dyes and Pigments*, Vol. 187, pp. 109123, 2021.

[39] K. Bhuvanewari, N. Nagasundaram, A. Lalitha, “Synthesis, anti-inflammatory activity, and molecular docking study of novel azo bis antipyrine derivatives against cyclooxygenase-2 enzyme”, *Journal of the Chinese Chemical Society*, Vol. 68, pp. 27-33, 2021.

[40] Y. Nuruki, H. Matsumoto, M. Tsukada, H. Tsukahara, T. Takajo, K. Tsuchida, K. Anzai, “Method to Improve Azo-Compound (AAPH)-Induced Hemolysis of Erythrocytes for Assessing Antioxidant Activity of Lipophilic Compounds”, *Chemical and Pharmaceutical Bulletin*, Vol. 69, pp. 67-71, 2021.