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Synthesis and antimicrobial evaluation of new heterocyclic compounds derived from 2-acetyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one

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Abstract:

The 2-acetyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one **2** prepared via acetylation of 5-methyl-1H-pyrazol-3-one **1** was reacted with aromatic aldehydes in 1:1 and 1:3 ratio to give chalcone derivatives **3a-c** and the tri-condensed product **6**. Chalcone derivative **3a** was condensed with hydrazine hydrate and hydroxylamine hydrochloride to give the corresponding bis pyrazole **4** and the oxime **5** derivatives, respectively. Also, it was reacted with bromine in 1:1 and 1:2 ratio to give the mono **7** and the dibromo **10** derivatives, respectively. Reaction of the monobromo derivative **7** with hydrazine hydrate and thiourea gave the pyrazolotriazine **8** and the 2-(thiazol-4-yl)-3-pyrazolone **9** derivatives, respectively. Compound **1** was reacted with chloroacetyl chloride to give the 2-(2-chloroacetyl)pyrazolone **11** derivative, which on reaction with hydrazine hydrate, urea and thiourea gave the 2-aminoglycyl pyrazolone **12** and the urea and thiourea derivatives **13a,b**. Furthermore, reaction of compound 1 with formaldehyde/piperidine, benzene sulfonyl chloride and chloroacetyl chloride gave the Mannich product **14**, the 2-phenylsulfonyl pyrazolone **15** and the bis pyrazolone **16**. All new compounds were prepared using both conventional and microwave techniques. Elemental analyses together with spectroscopic data including IR, ^{1}H-NMR in addition to ^{13}C-NMR and mass spectra submit proofs for the chemical structures for all compounds. Some of the new pyrazolone derivatives showed antimicrobial activities.

Keywords:

Pyrazolone, microwave, conventional, antibacterial, antifungal.

1. Introduction:

Pyrazolones and fused derivatives are considered one of the most important heterocyclic compounds due to their biological and pharmacological activities and their application in industrial chemistry [1-3]. Pyrazolones 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 method was practically superior to conventional heating method since it showed improvement in the yield and the time of the reactions and also they were considered as green chemistry. [23, 24]

From these findings and a continuation of our recent studies [25-32], this work aimed to investigate reaction of the 5-methyl-1H-pyrazol-3-one **1** [33] toward acetyl chloride to form 2-acetyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one **2**, which reacted with some aromatic aldehydes in different ratio to prepare novel chalcones **3a-c** and **6**. Compound **3a** reacted with hydrazine hydrate and hydroxylamine hydrochloride to form derivatives **4** and **5**, respectively. While, compound **2** reacted with bromine in ratio (1:1) [34] and (1:2) to form compounds **7** and **10**, respectively. However, compound 7 reacted with hydrazine hydrate and thiourea to form pyrazolotriazine and 2-(thiazol-4-yl)-3-pyrazolone derivatives **8** and **9**, respectively. Also, this work aimed to investigate the behavior and reactivity of compound **1** toward some carbon and nitrogen nucleophiles under different aspects in order to prepare novel heterocyclic compounds **11-16**.

On the other hand, comparison between conventional and microwave methods used in the preparation of different compounds by using different physical tools as YE, OE, AE, and RME. Elemental analyses together with spectroscopic data (IR, ^{1}H-NMR, ^{13}C-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. aeuroginosa). The antifungal of the compounds were tested against two fungi (C. albicans, A. flavus).

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 $60F_{254}$, BDH). All melting points were determined on a digital Stuart SMP3 electric melting point apparatus and are uncorrected. Microwave reactor Anton Par (monowave 300) was used for microwave irradiation reactions using borosilicate glass vials of 10 mL. Infrared (IR) spectra were measured on PerkinElmer 293 spectrophotometer (cm^{-1}) using KBr disks. ^{1}H-NMR and ^{13}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 ±0.4% of the theoretical values.

2.1.1. Synthesis of 2-acetyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (2)

A mixture of compound **1** (0.98 g, 0.01 mol.), acetyl chloride (0.78 mL, 0.01 mol.) in acetic acid (10 mL) was refluxed for 8h. The reaction mixture after cooling, was poured onto cold ice water (50 mL), the solid precipitated was filtered off, washed with water and crystallized from acetic acid to furnish compound **2**.

White crystals, m.p. 120-122 ^{O}C. IR (KBr) ν cm^{-1}: 1772, 1660 (C=O), 1616 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.07 (s, 3H, $\underline{CH_3}$-C=N), 2.48 (s, 3H, $\underline{CH_3}$-C=O), 3.78 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d6) δ (ppm): 11.61, 25.9, 62.5, 139.1, 161.4, and 168.2. MS: *m/z* 140 [M^+] (30.74%). Anal. Calcd. for $C_6H_8N_2O_2$ (140): C, 51.43; H, 5.71; N, 20.00. Found: C, 51.66; H, 5.54; N, 19.97.

2.1.2. General procedure for preparation compounds 3a-c

A mixture of compound **2** (1.4 g, 0.01 mol.), benzaldehyde (1.06 mL, 0.01 mol.), 2-hydroxy-3-methoxybenzaldehyde (1.52 g, 0.01 mol.) and/or 4-amino benzaldehyde (1.21 g, 0.01 mol.) in ethanol (30 mL) in presence of NaOH (0.4 g, 0.01 mol.) was refluxed for 5-8h. The reaction mixture after cooling was poured onto dil. HCl, the solid formed was collected by filtration, washed with water and recrystallized from methanol to give compounds **3a-c**, respectively.

2.1.2.1. 2-Cinnamoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (3a)

Brown crystals, m.p. 184-186 ^{O}C. IR (KBr) ν cm^{-1}: 1706 (C=O), 1596 (C=N), 1535 (C=C). ^{1}H-NMR (DMSO-d6) δ: 2.07 (s, 3H CH_3), 3.75 (s, 2H, CH_2), 7.00-7.60 (m, 7H, Ar-H & olefinic-H). ^{13}C-NMR (DMSO-d6) δ (ppm): 11.69, 62.5, 118.2, 120.3, 124.9, 126.0, 130.2, 138.7, 138.8, 139.8, 158.9, 161.2, and 163.8. MS: *m/z* 228 [M^+] (24.76%). Anal. Calcd. for $C_{13}H_{12}N_2O_2$ (228): C, 68.42; H, 5.26; N, 12.28. Found: C, 68.51; H, 5.18; N, 12.22.

2.1.2.2. 2-(3-(2-Hydroxy-3-methoxyphenyl)acryloyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (3b)

Green crystals, m.p. 160-162 ^{O}C. IR (KBr) ν cm^{-1}: 3577 (OH), 1716 (C=O), 1621 (C=N), 1576, 1537 (C=C). MS: *m/z* 274 [M^+] (30.75%). Anal. Calcd. for $C_{14}H_{14}N_2O_4$ (274): C, 61.31; H, 5.11; N, 10.21. Found: C, 61.15; H, 5.20; N, 10.09.

2.1.2.3. 2-(3-(4-Aminophenyl)acryloyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (3c)

Reddish brown crystals, m.p. 154-156 ^{O}C. IR (KBr) ν cm^{-1}: 3300-3100 (broad, NH_2), 1709 (C=O), 1588 (C=N), 1505 (C=C). MS: *m/z* 243 [M^+] (88.91%). Anal. Calcd. for $C_{13}H_{13}N_3O_2$ (243): C, 64.20; H, 5.35; N, 17.28;. Found: C, 64.31; H, 5.22; N, 17.14.

2.1.3. 3-Methyl-5'-phenyl-4',5'-dihydro-1'H-[1,3'-bipyrazol]-5(4H)-one (4)

A mixture of compound **3a** (2.28 g, 0.01 mol.), hydrazine hydrate (0.5 mL, 0.01 mol.) in acetic acid (15 mL) was refluxed for 4h. After cooling, the reaction mixture was poured onto cold ice water (50 mL), the solid that separated out was filtered off, washed with water and crystallized from methanol to give compound **4**.

Yellow Crystals, m.p. 230-232 ^{O}C. IR (KBr) ν cm^{-1}: 3210 (NH), 1658 (C=O), 1599, 1517 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.02 (s, 3H, CH_3), 3.45 (d, 2H, $\underline{CH_2}$-CHPh), 3.52 (s, 2H, $\underline{CH_2}$-C=O), 4.08 (d, 1H, $CH_2\underline{CH}$-Ph), 6.66-7.43 (m, 5H, Ar-H), 10.62 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d6) δ (ppm): 13.1, 42.07, 60.22, 69.9, 125.2, 127.4, 128.1, 128.9, 136.6, 146.5, 146.8, 159.5, and 161.8. MS: *m/z* 242 [M^+] (23.90%). Anal. Calcd. for $C_{13}H_{14}N_4O$ (242): C, 64.46; H, 5.78; N, 23.14. Found: C, 64.44; H, 5.87; N, 23.09.

2.1.4. 1-(5-(Hydroxyimino)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-3-phenylprop-2-en-1-one (5)

A mixture of compound **3a** (2.28 g, 0.01 mol.), hydroxylamine hydrochloride (0.69 mL, 0.01 mol.) in acetic acid (15 mL) was refluxed for 7h. After cooling, the reaction mixture was poured onto cold ice water (50 mL),

the solid formed was filtered off, washed with water and crystallized from butanol to give compound **5**.

Pale brown crystals, m.p. 250-252 ^{O}C. IR (KBr) ν cm^{-1}: 3400 (broad, OH), 1701 (C=O), 1599 (C=N), 1550 (C=C). ^{1}H-NMR (DMSO-d6) δ: 2.11 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 7.16-7.97 (m, 7H, Ar-H & olefinic-H), 11.27 (s, 1H, OH). MS: *m/z* 243 [M^+] (30.44%). Anal. Calcd. for $C_{13}H_{13}N_3O_2$ (243): C, 64.20; H, 5.35; N, 17.28. Found: C, 64.10; H, 5.49; N, 17.22.

2.1.5. 4-(Benzylidene)-2-cinnamoyl-5-(styryl)-2,4-dihydro-3H-pyrazol-3-one (6)

A mixture of compound **2**(1.4 g, 0.01 mol.), benzaldehyde (3.18 mL, 0.03 mol.) in ethanol (30 mL) in the presence of NaOH (0.4 g, 0.01 mol.) was refluxed for 12h. The reaction mixture after cooling was poured onto dil. HCl, the solid formed was collected by filtration, washed with water and recrystallized from methanol to give compound **6**.

Red crystals, m.p. 210-212 ^{O}C. IR (KBr) ν cm^{-1}: 1677 (C=O), 1598 (C=N). ^{1}H-NMR (DMSO-d6) δ: 6.35 (d, 1H, C=CHPh), 7.00-7.90 (m, 19H, Ar-H & olefinic-H). ^{13}C-NMR (DMSO-d6) δ (ppm): 126.3, 127.2, 128.5, 129.0, 129.2, 129.3, 129.5, 129.9, 130.6, 131.5, 133.9, 134.7, 137.8, 158.7, 158.8, 158.9, and 161.7. MS: *m/z* 404 [M^+] (27.32%). Anal. Calcd. for $C_{27}H_{20}N_2O_2$ (404): C, 80.20; H, 4.95; N, 6.93. Found C, 80.09; H, 5.07; N, 6.88.

2.1.6. 2-(2-Bromoacetyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (7)

To solution of compound **2** (1.4 g, 0.01 mol.) in glacial acetic acid (25 mL), bromine (1.58 mL, 0.01 mol.) was added dropwise with continuous stirring. The reaction mixture was stirred for another 3h then poured onto cold water and the solid formed was collected by filtration, washed with water and crystallized from ethanol to give compound **7**.

Yellow crystals, m.p. 288-290 ^{O}C. IR (KBr) ν cm^{-1}: 1721, 1657 (C=O), 1607 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.17 (s, 3H, CH_3), 2.49 (s, 2H, $\underline{CH_2}$-C=O), 4.43 (s, 2H, CO$\underline{CH_2}$Br). ^{13}C-NMR (DMSO-d6) δ (ppm): 12.1, 29.2, 69.9, 137.6, 170.2, and 173.3. MS: *m/z* 217 [M^+] (36.37%). Anal. Calcd. for $C_6H_7N_2O_2Br$ (217): C, 33.18; H, 3.23; N, 12.90; Br, 36.40. Found: C, 33.01; H, 3.17; N, 12.98; Br, 36.29.

2.1.7. 7-Methyl-2,8-dihydropyrazolo[5,1-c][1,2,4]triazin-4(3H)-one (8)

A mixture of compound **7** (2.17 g, 0.01 mol.), hydrazine hydrate (0.5 mL, 0.01 mol.) in acetic acid (15 mL) was refluxed for 6h. The reaction mixture after cooling, was poured onto ice water (50 mL), the solid that separated out was filtered off, washed with water and crystallized from methanol to give compound **8**.

Pale yellow powder, m.p. 240-242 ^{O}C. IR (KBr) ν cm^{-1}: 3098 (NH), 1655 (C=O), 1604, 1530 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.06 (s, 3H, CH_3), 3.08 (s, 2H, CH_2), 3.61 (s, 2H, CO$\underline{CH_2}$NH), 8.99 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d6) δ (ppm):14.0, 22.6, 101.8, 158.1, 158.2, and 161.2. MS: *m/z* 152 [M^+] (22.85%). Anal. Calcd. for $C_6H_8N_4O$ (152): C, 47.37; H, 5.26; N, 36.84. Found: C, 47.55; H, 5.10; N, 36.73.

2.1.8. 2-(2-Aminothiazol-4-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (9)

A mixture of compound **7** (2.17 g, 0.01 mol.), thiourea (0.76 mL, 0.01 mol.) in acetic acid (15 mL) was refluxed for 8h. The reaction mixture after cooling, was poured onto ice water (50 mL), the solid that separated out was filtered off, washed with water and crystallized from acetone to give compound **9**.

Gray powder, m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 3384, 3165 (NH_2), 1771 (C=O), 1603 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.07 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 7.06 (s, 1H, Ar-H), 7.19 (s, 2H, NH_2, D_2O exchangeable). MS: *m/z* 196 [M^+] (79.81%). Anal. Calcd. for $C_7H_8N_4OS$ (196): C, 42.86; H, 4.08; N, 28.57; S, 16.32. Found: C, 42.99; H, 3.95; N, 28.49; S, 16.39.

2.1.9. 2-Bromo-1-(4-bromo-5-hydroxy-3-methyl-1H-pyrazol-1-yl)ethan-1-one (10)

To solution of compound **2** (1.4 g, 0.01 mol.) in acetic acid (30 mL), bromine (3.18 mL, 0.02 mol.) was added dropwise with continuous stirring. The reaction mixture was stirred for another 3h, then poured onto ice water (300 mL). The solid formed was filtered off, washed with water and crystallized from butanol to give compound **10**.

Orange crystals, m.p. 218-220 ^{O}C. IR (KBr) ν cm^{-1}: 3353 (OH), 1722 (C=O), 1605 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.23 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 12.05 (s, 1H, OH, D_2O exchangeable). MS: *m/z* 295 [M^+] (19.41%). Anal. Calcd. for $C_6H_6N_2O_2Br_2$ (295): C, 24.41; H, 2.03; N, 9.49; Br, 53.56. Found: C, 24.53; H, 1.94; N, 9.54; Br, 53.47.

2.1.10. 2-(2-Chloroacetyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (11)

A mixture of compound **1** (0.98 g, 0.01 mol.), chloro acetyl chloride (1.13 mL, 0.01 mol.) in dry acetone (20 mL) in the presence of anhydrous K_2CO_3 (1.38 g, 0.01 mol.) was refluxed for 7h. The solid obtained after filtration while hot and cooling was collected by filtration, washed with water and recrystallized from acetone to give compound **11**.

Reddish brown crystals, m.p. 174-176 ^{O}C. IR (KBr) ν cm^{-1}: 1702, 1652 (C=O), 1566 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.14 (s, 3H, CH_3), 3.68 (s, 2H, $\underline{CH_2}$CO), 5.47 (s, 2H, $\underline{CH_2}$Cl). ^{13}C-NMR (DMSO-d6) δ (ppm): 11.6, 25.9, 89.9, 159.7, 163.2, and 165.4. MS: *m/z* 174 [M^+] (7.28%). Anal. Calcd. for $C_6H_7N_2O_2Cl$ (174): C, 41.38; H, 4.02; N, 16.09; Cl, 20.40. Found: C, 41.45; H, 3.99; N, 15.97; Cl, 20.57.

2.1.11. 2-(Aminoglycyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (12)

A mixture of compound **11** (1.74 g, 0.01 mol.) and hydrazine hydrate (0.5 mL, 0.01 mol.) was fused for 5h. The reaction mixture after cooling was washed with ethanol and recrystallized from butanol to give compound **12**.

Brown crystals, m.p. 254-256 ^{O}C. IR (KBr) ν cm^{-1}: 4500-2000 (broad, NH_2&NH), 1680, 1665 (C=O), 1527 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.12 (s, 3H, CH_3), 2.55 (s, 2H, $\underline{CH_2}$-C=O), 2.63 (s, 2H, $\underline{CH_2}$-NH), 5.32 (s, 2H, NH_2, D_2O exchangeable), 8.07 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d6) δ (ppm): 11.3, 22.8, 89.9, 157.0, 158.7, and 162.3. MS: *m/z* 170 [M^+] (100%). Anal. Calcd. for $C_6H_{10}N_4O_2$ (170): C, 42.35; H, 5.88; N, 32.94. Found: C, 42.24; H, 6.01; N, 33.04.

2.1.12. General procedure for preparation compounds 13a,b

A mixture of compound **11** (1.74 g, 0.01 mol.), urea (0.60 g, 0.01 mol.) and/or thiourea (0.76, 0.01 mol.) in dioxane (10 mL) was refluxed for 6-8 h. The solid obtained after cooling was collected by filtration, washed with water and recrystallized from methanol to give compounds **13a** and **13b**, respectively.

2.1.12.1. 1-(2-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)urea (13a)

Brown crystals, m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 3433, 3342 (NH_2), 3209 (NH), 1709, 1664 (C=O), 1623 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.02 (s, 3H, CH_3), 3.32 (s, 2H, $\underline{CH_2}$-C=O), 4.11 (s, 2H, $\underline{CH_2}$-NH), 5.52 (s, 2H, NH_2, D_2O exchangeable), 8.96 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 198 [M^+] (18.93%). Anal. Calcd. for $C_7H_{10}N_4O_3$ (198): C, 42.42; H, 5.05; N, 28.28. Found: C, 42.33; H, 4.94; N, 28.30.

2.1.12.2. 1-(2-(3-Methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)thiourea (13b)

Brown crystals, m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 3364, 3251 (NH_2), 3154 (NH), 1710 (C=O), 1610 (C=N), 1250 (C=S). MS: *m/z* 214 [M^+] (31.39%). Anal. Calcd. for $C_7H_{10}N_4O_2S$ (214): C, 39.25; H, 4.67; N, 26.17; S, 14.95. Found: C, 39.17; H, 4.80; N, 26.29; S, 14.88.

2.1.13. 5-Methyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-pyrazol-3-one (14)

A mixture of compound **1** (0.98 g, 0.01 mol.), formaldehyde (0.3 mL, 0.01 mol.) and piperidine (0.85 mL, 0.01 mol.) in dioxane (10 mL) was refluxed for 3h. The reaction mixture after cooling, was poured onto ice water (50 mL), the solid formed was filtered off, washed with water and crystallized from methanol to produce compound **14**.

Gray crystals, m.p. 190-192 ^{O}C. IR (KBr) ν cm^{-1}: 1703 (C=O), 1611 (C=N). ^{1}H-NMR (DMSO-d6) δ: 1.40-1.54 (m, 6H, $(CH_2)_3$), 1.94 (s, 3H, CH_3), 2.50 (t, 4H, $2CH_2$), 3.94 (s, 2H, $\underline{CH_2}$CO), 4.57 (s, 2H, N$\underline{CH_2}$N). MS: *m/z* 195 [M^+] (100%). Anal. Calcd. for $C_{10}H_{17}N_3O$ (195): C, 61.54; H, 8.72; N, 21.54. Found: C, 61.44; H, 8.89; N, 21.41.

2.1.14. 5-Methyl-2-(phenylsulfonyl)-2,4-dihydro-3H-pyrazol-3-one (15)

A mixture of compound **1** (0.98 g, 0.01 mol.), benzene sulphonyl chloride (1.76 mL, 0.01 mol.) in dioxane (15 mL) was refluxed for 7h. The reaction mixture after cooling, was poured onto ice water (50 mL), the obtained solid was filtered off, washed with water and crystallized from methanol to give compound **15**.

Beige crystals, m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 1657 (C=O), 1639 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.09 (s, 3H, CH_3), 3.57 (s, 2H, CH_2), 7.31-7.65 (m, 5H, Ar-H). ^{13}C-NMR (DMSO-d6) δ (ppm): 12.3, 26.7, 125.6, 127.8, 128.8, 147.8, 151.2, and 161.4. MS: *m/z* 238 [M^+] (70.91%). Anal. Calcd. for $C_{10}H_{10}N_2O_3S$ (238): C, 50.42; H, 4.20; N, 11.76; S, 13.44. Found: C, 50.30; H, 4.29; N, 11.88; S, 13.32.

2.1.15. 2,2'-(1-Oxoethane-1,2-diyl)bis(5-methyl-2,4-dihydro-3H-pyrazol-3-one) (16)

A mixture of compound **1** (1.96 g, 0.02 mol.), chloroacetyl chloride (1.13 mL, 0.01 mol.) in dry dioxane (10 mL) was refluxed for 12h. The obtained solid after cooling was collected by filtration, washed with ethanol and recrystallized from butanol to give compound **16**.

Black powder, m.p. 164-166 ^{O}C. IR (KBr) ν cm^{-1}: 1727 (C=O), 1645 (C=N). ^{1}H-NMR (DMSO-d6) δ: 2.16 (s, 6H, $2CH_3$), 2.49 (s, 4H, $2CH_2$), 4.13 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d6) δ (ppm): 11.3, 39.7, 63.8, 143.9, 159.3, and 166.8. MS: *m/z* 236 [M^+] (28.03%). Anal. Calcd. for $C_{10}H_{12}N_4O_3$ (236): C, 50.85; H, 5.08; N, 23.73. Found: C, 50.91; H, 5.15; N, 23.60.

2.2. Comparison between microwave and conventionalmethods

In the microwave reactions, the same reactants amounts in the conventionaltechnique were used. The reaction completion was illustrated by using TLC. The reaction mixtures were washed with ethanol and crystallized from the suitable solvent. The conventionaland microwave reaction times were showed in (Table 1). The comparison in terms of yields and times between the prepared compounds by using conventionaland microwave techniques were reported. However, we used the yield economy (YE) as a term to determine the conventionaland microwave synthetic different efficiencies of the same reaction. Calculation of YE was occurred through: $YE = \frac{yield\%}{Reaction\ time\ "min"}$.

In this report, the YE was used to provide the yields obtained conclusively enhanced under microwave and conventionalconditions. The equation of RME is: $\mathrm{RME} = \frac{Wt\ of\ isolateted\ product}{Wt\ of\ reactants}$.

While, OE was used for the direct comparisons between the two reaction types and can be calculated through $OE = \frac{RME}{AE} x\ 100$. 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 conventionaland 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. Show the comparison in terms of physical data between the synthesized compounds under conventionaland microwave techniques.

Cpd. no.	Time "min"		Yield %		YE		RME		OE		AE
	Th.	M.W.	Th.	M.W.	Th.	M.W.	Th.	M.W.	Th.	M.W.	
2	480	2	67	92	0.1396	46	67.34	81.52	76.00	92.00	88.61
3a	480	3	71	90	0.1479	30	39.38	49.93	63.04	79.93	62.47
3b	300	1	65	94	0.2167	94	42.57	56.36	63.85	84.54	66.67
3c	360	2	68	93	0.1889	46.50	38.79	53.04	60.66	82.94	63.95
4	240	3	69	89	0.2875	29. 67	49.40	66.59	56.75	76.50	87.05
5	420	4	70	91	0.1667	22.75	47.65	61.94	58.24	75.70	81.82
6	720	6	71	88	0.0986	14.67	46.04	57.07	65.76	81.52	70.01
7	180	4	71	89	0.3944	22.25	48.75	61.12	57.51	72.10	84.77
8	360	1	66	87	0.1833	87	30.68	40.44	53.90	71.05	56.92
9	480	2	62	88	0.1292	44	34.42	48.86	51.46	73.05	66.89
10	300	1	64	92	0.2133	92	39.83	57.26	55.89	80.35	71.26
11	420	1	67	90	0.1595	90	28.64	38.48	57.44	77.18	49.86
12	300	3	64	90	0.2133	30	48.57	68.30	64.00	90.00	75.89
13a	480	2	63	91	0.1313	45.5	38.03	54.93	46.10	66.58	82.50
13b	360	1	67	88	0.1861	88	42.42	55.72	49.56	65.09	85.60
14	180	3	68	92	0.3778	30.67	42.36	57.32	48.88	66.14	86.67
15	420	3	62	91	0.1476	30.3 3	40.76	59.83	46.93	68.88	86.86
16	720	5	70	93	0.0972	18.60	41.61	55.28	54.48	72.37	76.38

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 aeuroginosa). 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. aeuroginosa, C.albicans and A. flavus. The petri dishes were incubated at 36 ^{O}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:

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

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 [30, 31]. 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 **2, 3c, 8** and **13b**, while the most active compounds were **2, 11** and **13b** for Gram-negative and **2, 3c, 8** and **13b** for Gram-positive bacteria. In addition, Gram-positive bacteria were more sensitive to the compounds compared with Gram-negative ones (Figures 1 and 2).

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

Cpd.	E. coli		P. aeuroginosa		S. aureus		B. subtilis		C. Albicans		A. flavus	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
2	13	52	18	78.3	16	66.7	19	82.6	16	59.2	18	72
3a	NA	----	NA	----	NA	----	3	13	NA	----	2	8
3b	NA	----	NA	----	NA	----	NA	----	NA	----	5	20
3c	15	60	18	78.3	17	70.8	20	86.9	21	77.8	19	76
4	7	28	10	43.5	11	45.8	13	56.5	6	22.2	12	48
5	NA	----	NA	----	4	16.7	6	26.1	NA	----	7	28
6	3	12	7	30.4	8	33.3	10	43.5	4	14.8	11	44
7	NA	----	6	26.1	7	29.2	8	34.8	NA	----	NA	----
8	10	40	15	65.2	14	58.3	16	69.6	14	51.8	17	68
9	NA	----	NA	----	NA	----	NA	----	NA	----	NA	----
10	NA	----	4	17.4	5	20.8	7	30.4	NA	----	NA	----
11	11	44	15	65.2	13	54.2	16	69.6	10	37	15	60
12	7	28	10	43.5	12	50	14	60.9	8	29.6	14	56
13a	8	32	12	52.2	12	50	14	60.9	11	40.7	16	64
13b	19	76	22	95.6	18	75	21	91.3	25	92.6	23	92
14	NA	----	2	8.7	NA	----	4	17.4	NA	----	NA	----
15	5	20	8	34.8	10	41.7	10	43.5	3	11.1	9	36
16	9	36	14	60.9	12	50	15	65.2	8	29.6	13	52
Ampicillin	25	100	23	100	24	100	23	100	NA	----	NA	----
Colitrimazole	NA	----	NA	----	NA	----	NA	----	27	100	25	100

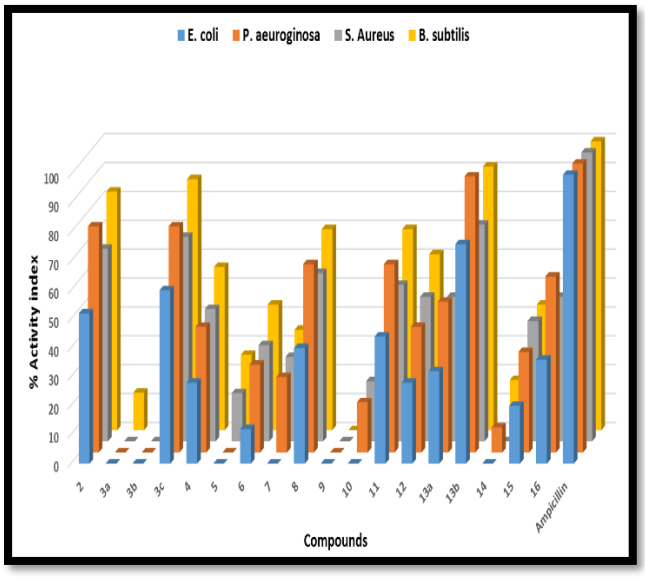


Fig (1): % activity of most potent compounds against bacteria.

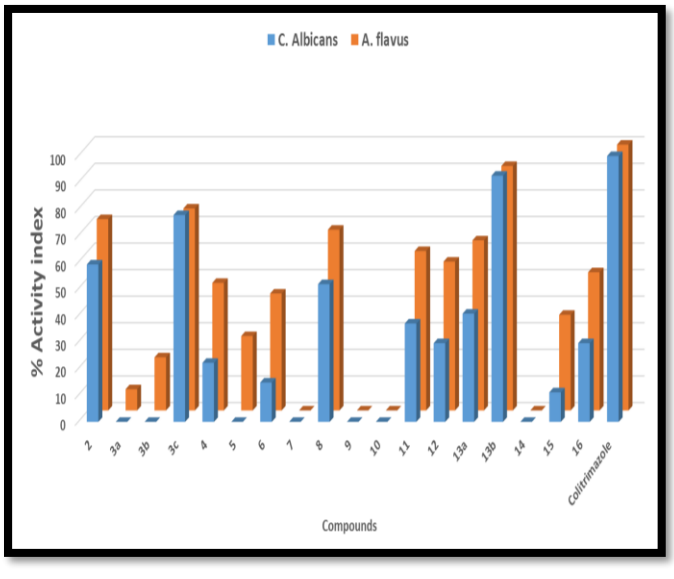


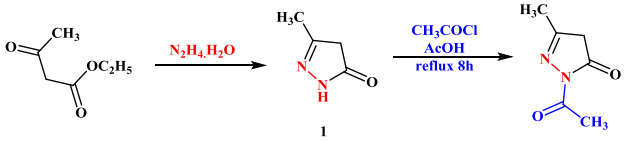
Fig (2): % activity index of most potent compounds against fungal.

3. Results and discussion:

3.1. Synthesis:

The new pyrazolone derivatives were prepared following the reaction sequences depicted in Schemes 1-4. The starting material pyrazolone **1** was prepared by literature known procedure [33] using ethyl acetoacetate and hydrazine hydrate.

at υ 1660 cm^{-1}. Its ^{1}H-NMR showed peaks of the three protons of acetyl group at δ 2.48 ppm. Its ^{13}C-NMR showed peaks of 2 C=O at δ 161.4 and 168.2 ppm. Its mass spectrum revealed the molecular ion peak at *m/z* 140 (30.74%). (Scheme 1)



Scheme 1. Synthesis of the acetyl derivative 2.

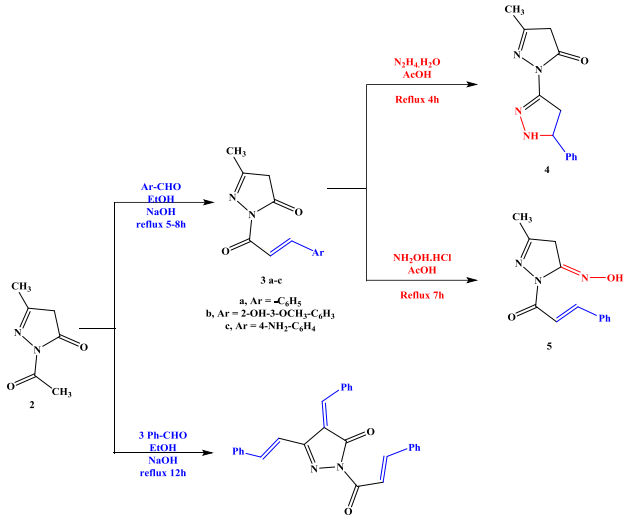
Treatment of compound **2** with benzaldehyde, 2-hydroxy-3-methoxybenzaldehyde and/or 4-amino benzaldehyde in 1:1 ratio in ethanoic solution of NaOH under Claisen Schmidt conditions afforded the chalcone derivatives **3a-c**, respectively. The structure of the obtained derivatives **3a-c** were confirmed from their spectroscopic data and elemental analysis.

Compound **3a** can be used as a key intermediate for the preparation of new heterocyclic rings via its reactions with different nitrogen nucleophiles such as hydrazine hydrate, and hydroxylamine hydrochloride to form compounds **4** and **5**, respectively. Thus, reaction of compound **3a** with hydrazine hydrate gave 3-methyl-5'-phenyl-4',5'-dihydro-1'H-[1,3'-bipyrazol]-5(4H)-one **4** via β-attack on the C=C moiety in compound **3a** by 1,5-dipolar cyclization to give the pyrazole ring. The structure of compound **4** was confirmed from its IR spectrum displayed band of NH at υ 3210 cm^{-1}. Its ^{1}H-NMR spectrum revealed signals at δ 10.62 ppm corresponding to NH. Its mass spectrum showed a molecular ion peak at *m/z*=242 (23.90%) which corresponding to molecular formula $C_{13}H_{14}N_4O$.

Similarly, compound **3a** when allowed to react with hydroxylamine hydrochloride gave the corresponding compound **5**. The structure of compound **5** was illustrated from its IR spectrum displayed band of OH at υ 3400 cm^{-1}. Its ^{1}H-NMR spectrum revealed band at δ 11.27 ppm corresponding to OH. Its mass spectrum revealed a molecular ion peak at *m/z*=243 (30.44%) which corresponding to molecular formula $C_{13}H_{13}N_3O_2$.

Interestingly, reaction of compound **2** with benzaldehyde in 1:3 ratio afforded the tri condensed product **6**, its mass spectrum showed the molecular ion peak at *m/z*= 404 (27.32%) which coincide with the molecular weight supporting the proposed identity of the structure. (Scheme 2)

Acetylation of compound **1** with acetyl chloride afforded 2-acetyl-3-pyrazolone derivative **2.** The structure of the acetylated compound **2** was illustrated form its IR spectrum which displayed bands of C=O at υ 1772 cm^{-1} and pyrazolone C=O



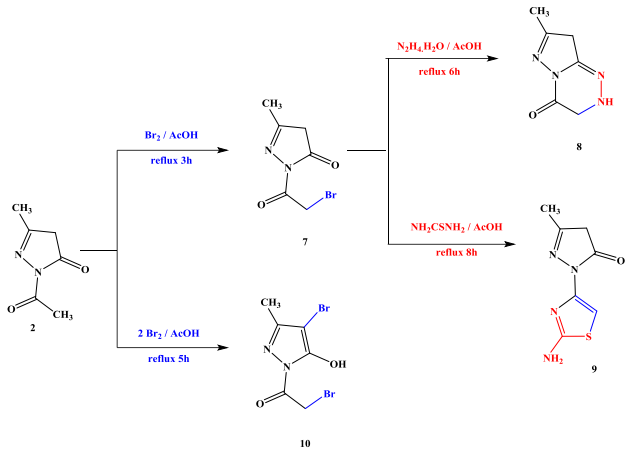
Scheme 2. Synthesis of pyrazolone derivatives 3-6.

The N-acetyl derivative **2** readily underwent bromination when treated with bromine in 1:1 ratio in acetic acid solution to give the N-α-bromoacetyl pyrazolone derivative **7**. The structure of compound **7** was confirmed form its IR spectrum displayed bands of 2C=O at υ 1770 & 1657 cm^{-1}. Its ^{1}H-NMR spectrum revealed signals at δ 4.43 ppm corresponding to CO$\underline{CH_2}$Br. Its mass spectrum showed a molecular ion peak at *m/z*=217 (36.37%) which corresponding to molecular formula $C_6H_7N_2O_2Br$.

Compound **7** can be used to prepare the annulated compound **8** via its reaction with hydrazine hydrate through elimination one molecule of HBr and one molecule of water. The structure of pyrazolotriazinone **8** was confirmed form its IR spectrum displayed bands of NH & C=O at υ 3098 & 1655 cm^{-1}, respectively. Its ^{1}H-NMR spectrum showed peaks at δ 8.99 ppm corresponding to NH. Its mass spectrum revealed a molecular ion peak at *m/z*=152 (22.85%) which corresponding to molecular formula $C_6H_8N_4O$.

On the other hand, reaction of compound **7** with thiourea gave the thiazole derivative **9** which is in accordance with a previous publication [34]. The structure of thiazole **9** was confirmed form its IR spectrum displayed band of NH_2 at υ 3384, 3165 cm^{-1}. Its mass spectrum revealed a molecular ion peak at *m/z*=196 (79.81%) which corresponding to molecular formula $C_7H_8N_4OS$.

Interestingly, reaction of compound **2** with bromine in 1:2 ratio gave compound **10**. Its mass spectrum showed its molecular ion peak *m/z*= 295 (19.41%), which agreed with the proposed structure. (Scheme 3)



Scheme 3. Synthesis of pyrazolone derivatives 7-10.

While, 2-(2-chloroacetyl)-pyrazolone derivative **11** was obtained via the reaction of compound **1** with chloroacetyl chloride in 1:1 molar ratio. The structure of the chloroacetyl pyrazolone **11** was confirmed form its ^{1}H-NMR showed peaks of the two protons of CO$\underline{CH_2}$Cl group at δ 5.47 ppm. Its ^{13}C-NMR showed peaks of 2 C=O at δ 163.2 and 165.4 ppm. Its mass spectrum revealed the molecular ion peak at *m/z*= 174 (7.28%) which corresponding to molecular formula $C_6H_7N_2O_2Cl$.

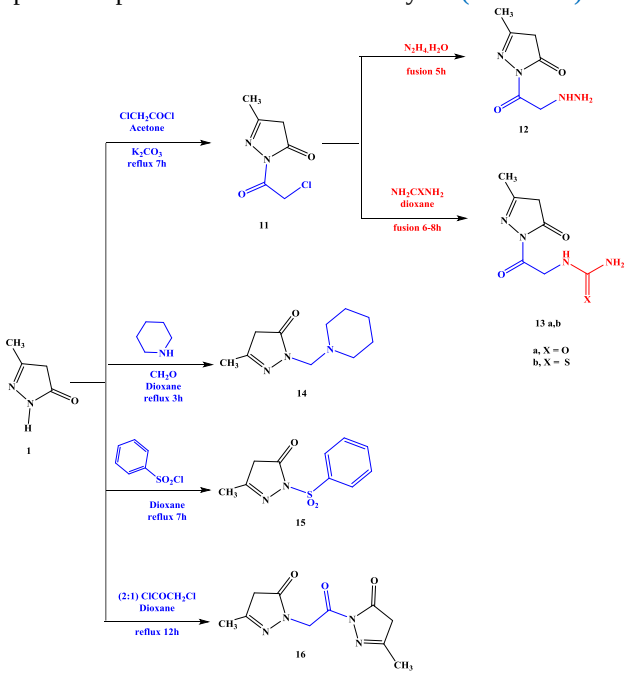
Reaction of compound **11** toward nitrogen nucleophiles namely hydrazine hydrate, urea and/or thiourea have been investigated and afforded compounds **12**, **13a** and **13b**, respectively. The structure of the hydrazide derivative **12** was illustrated form its IR spectrum displayed bands of NH_2 and NH at υ 4500-200 cm^{-1} and 2C=O at υ 1680, 1665 cm^{-1}. Its ^{1}H-NMR showed peaks of NH_2 and NH at δ 5.32 and 8.07 ppm, respectively. Its ^{13}C-NMR showed peaks of C=O at δ 158.7 and 162.3 ppm. Its mass spectrum revealed the molecular ion peak at *m/z*= 170 (100%) which corresponding to molecular formula $C_6H_{10}N_4O_2$.

While, the structure of the urea derivative **13a** was confirmed form its IR spectrum displayed bands of NH_2 at υ 3433, 3342 cm^{-1}, NH at υ 3209 cm^{-1} and 2C=O at υ 1709, 1664 cm^{-1}. Its ^{1}H-NMR showed protons of NH_2 and NH at δ 5.52 and 8.96ppm, respectively. Its mass spectrum revealed the molecular ion peak at *m/z*= 198 (18.93%) which corresponding to molecular formula $C_7H_{10}N_4O_3$. Also, the structure of the thiourea derivative **13b** was illustrated form its IR spectrum displayed bands of NH_2 at υ 3364, 3251 cm^{-1}, NH at υ 3154 cm^{-1}, C=O at υ 1710 cm^{-1} and C=S at υ 1250 cm^{-1}. Its mass spectrum revealed the molecular ion peak at *m/z*= 214 (31.39%) which corresponding to molecular formula $C_7H_{10}N_4O_2S$.

On the other hand, Mannich reaction of compound 1 with a mixture of formalin and piperidine as monobasic secondary amine in a molar ratio (1:1:1) afforded 2-(piperidin-1-ylmethyl)-3-pyrazolone derivative **14**. The structure of compound **14** was illustrated form its IR spectrum displayed broad bands of C=O at υ 1703 cm^{-1} and C=N at υ 1611 cm^{-1}. Its mass spectrum revealed the molecular ion peak at *m/z*= 195 (100%) which corresponding to molecular formula $C_{10}H_{17}N_3O$.

However, benzene sulphonyl derivative **15** was prepared through reaction of compound **1** with benzene sulphonyl chloride. The structure of compound **15** was confirmed form its IR spectrum displayed C=O at υ 1657 cm^{-1}. Its ^{1}H-NMR showed peaks of the five aromatic protons at δ 7.31-7.65 ppm. Its ^{13}C-NMR showed peaks of C=O at δ 161.4 ppm. Its mass spectrum revealed the molecular ion peak at *m/z*= 238 (70.91%) which corresponding to molecular formula $C_{10}H_{10}N_2O_3S$.

Interestingly, when the reaction of compound **1** with chloroacetyl chloride was carried out in 2:1 ratio the bis-pyrazolone derivative **16** was obtained through elimination of two molecules of HCl. The structure of the obtained derivative **16** was confirmed from its spectroscopic data and elemental analysis. (Scheme 4)



Scheme 4. Synthesis of pyrazolone derivatives 11-16.

3.2. Antimicrobial activity:

The new synthesized pyrazolone derivatives antimicrobial activities were determined using the inhibition zone diameter method in term % Activity index against two gram positive bacteria (S. aureus, B. subtilis), two gram negative bacteria (E. coli, P. aeuroginosa), and two fungi (C. albicans, A. flavus). (Table 1) show inhibition zone diameters afforded by using the new synthesized pyrazolones as antimicrobial agents via gram negative and gram positive bacterial strains. Generally, the obtained antimicrobial activities in terms of % Activity index for the desired derivatives are high in case of fungi and bacteria. That revealed higher antimicrobial activities for the tested pyrazolones against the different types of fungi and bacteria. The bacteria cell membrane was created form a dense wall comprising many teichoic acid and peptidoglycan layers (Figure 3) connected with glycerol

ribitol "polyhydric alcohol" via a phosphorus bonds and surrounded with lipopolysaccharides and some of proteins.

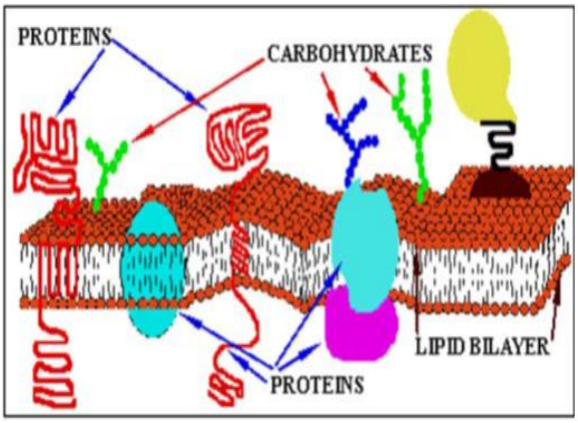


Fig (3): The bacterial outer cell membrane structure.

The action mode of the prepared heterocyclic biocides against the microorganisms either bacteria and/or fungi were reported as the biocides adsorption mechanism on the microorganism outer cell membrane because of the adsorptive characteristic over the heteroatoms. According to "gram positive" bacteria, the biocidal molecules adsorption take place on the lipoteichonic layer which was characterized by a charged nature and by the ability toward the biocide molecules heteroatoms. However in "gram negative" bacteria, the lipid layer is highly nonpolar layer toward the biocide molecules target. The results recorded in (Table 1) showed that the new synthesized compounds antimicrobial activity were lower against the gram positive bacteria than their activities against the gram negative bacterial strains. Thus, that can be refer to the outer cell membranes chemical nature of both bacterial type Gram positive bacteria characterized. Gram negative bacteria were characterized by the rigid, and cross linking cell wall, whose resists the different biocides penetration into the core of the cell.

On the contrary, the gram positive bacteria have very thin cell membrane whose allow the biocides penetration into core of the cell. The gram negative bacteria were characterized by the rigid cell membrane, whose resist the different biocide molecules penetration into the cells. The new synthesized pyrazolone molecules action mode were suggested by an adsorption action mode. The different new molecules owe heteroatoms containing lone pairs of electron including nitrogen, sulfur, and/or oxygen atoms in addition to the double bonds and phenyl rings. The electron rich molecules were adsorbed on the outer cell membrane of the opposite charged cites and being to penetrate to this membrane. So, the penetrated molecules (**2, 3c, 8** and **13b**) into the core of the cell started inside the cells many actions including proteins denaturation and complexation by DNA inside the bacterial nucleus. These interactions were disturbed the microorganisms biological activities and were lead the bacterial cells death. The different compounds adsorption tendencies were determined their adsorption ability on the cellular membranes which is dependent on their chemical structures. [35, 36]

4. Conclusion:

Pyrazolone derivative **1** was used as a versatile material for the synthesis of different derivatives such as N-acetylpyrazolone, chalcones, bipyrazole, 5-hydroxyimino-pyrazole, tribenzylidene pyrazolone, 2-bromoacetyl pyrazolone, pyrazolotriazinone, N-thiazolylpyrazolone, dibromo pyrazolone, N- aminoglycyl pyrazolone, urea, thiourea, Mannish product, N-benzenesulphonyl, bispyrazolone through the reaction with different electrophiles and nucleophiles. The new compounds structures were established from spectroscopic data. All the newly synthesized compounds were prepared by conventional method and under microwave irradiation. The newly synthesized compounds were tested against Gram-positive and Gram-negative bacteria, and fungi.

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

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