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Synthesis and biological activity of a new class of enaminonitrile pyrazole

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Abstract

The present work illustrates the treatment of 5-amino-1,3-diphenyl-1H-pyrazole-4-carbonitrile **(1)** with maleic, phthalic anhydrides, acetyl chloride, and benzene sulfonyl chloride to afford the pyrazole derivatives **2-5**. The treatment of pyrazole derivative **1** with some active methylene reagents namely: malononitrile, cyanoacetamide, ethyl acetoacetate afforded the pyrazolopyridine **6-9**. Reaction of compound **1** with acetic anhydride gave the pyrazolopyrimidinone **10**, which was allowed to react with P_2S_5 and $POCl_3$ to give the thione **11** and the chloro **12** derivatives. The reaction of compounds **11** and **12** with thiosemicarbazide under different conditions gave **13** and **14**. Compound **12** when reacted with hydrazine hydrate and p-toluidine gave compounds **15** and **16**. The reaction of compound **10** with ethyl chloroacetate gave compound **17** which was allowed to react with thiosemicarbazide to give **22** and also to consecutive reactions with hydrazine hydrate, carbon disulfide, piperidine, hydrazine hydrate to give compounds **18-21**. Finally, compound **1** was reacted with chloroacetic acid, hydrated hydrazine, urea, thiourea, benzaldehyde and triethylamine to give compounds **23-29**. The newly prepared compounds were characterized by using IR, ^{13}C-NMR, ^{1}H-NMR, and mass spectral data. Some new pyrazole derivatives showed highly antibacterial activities.

Keywords: Pyrazole, pyrazolopyrimidine, pyrazolopyridine, antibacterial.

1. Introduction:

A large number of pyrazole and their fused derivatives have different biological activities such as antimicrobial [1], anticancer [2], antiviral [3], analgesic [4], antagonist [5], anti-inflammatory [6], herbicidal [7] anthelmintic [8], antioxidant [9], insecticidal, acaricidal [10] and antimitotic activities [11].

The above mentioned screening biological activities facts motivate our concerning [12-15] for the preparation of various new products comprise pyrazole moiety and were tested as antimicrobial agents against different types of fungi and bacteria.

2. Experimental

2.1. Chemistry:

Commercially available reagents solvents starting and materials were used without purification. All the melting points have been measured on the electric digital Stuart melting point apparatus "SMP3". The infrared spectra have been recorded using KBr disks on "PerkinElmer-293 infrared spectrophotometer". ^{1}H-NMR and ^{13}C-NMR spectra have been recorded at 300 MHz on a "Varian Mercury spectrometer" using TMS as an internal standard in using DMSO-d_6. Chemical shift "δ" in ppm and coupling constants "J" in Hz. The mass spectra (M.S) were measured on the Shimadzu Gas chromatography mass spectrometer "GC-2010" at 70 eV. Elemental microanalyses were measured on a PerkinElmer analyzer "CHN-2400". While the microanalyses were within ± 0.4% comparative to the theoretical values. The products biological activities were evaluated at The Regional Center for Biotechnology and Mycology (RCMB), Al- Azhar University. All chemical reactions were monitored by TLC.

2.1.1. Formation of 5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-1,3-diphenyl-1H-pyrazole-4-carbonitrile (2)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in acetic acid (20 mL), maleic anhydride (0.98 g, 0.01 mol.) was added. The mixture was refluxed for 5h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from methanol gave compound **2**.

Yield 75%; pale yellow crystal; m.p. 180-182 ^{O}C; IR (KBr, υ, cm^{-1}) 2228 (CN), 1725, 1659

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(CO), 1593 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 6.74-7.86 (m, 12H; Ar-H); MS: *m/z* 340 (M^+) (30.6%). Anal. Calcd. for $C_{20}H_{12}N_4O_2$: C, 70.58; H, 3.55; N, 16.46; Found: C, 71.02; H, 3.25; N, 16.16%.

2.1.2. Formation of 5-(1,3-dioxoisoindolin-2-yl)-1,3-diphenyl-1H-pyrazole-4-carbonitrile (3)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in acetic acid (15 mL), phthalic anhydride (1.3 g, 0.01 mol.) was added. The mixture was refluxed for 5h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol afforded compound **3**.

Yield 73%; red crystal; m.p. 110-112 ^{O}C; IR (KBr, *υ*, cm^{-1}) 2227 (CN), 1784, 1725 (CO), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.75-8.54 (m, 14H; Ar-H); MS: *m/z* 390 (M^+) (38.5%). Anal. Calcd. for $C_{24}H_{14}N_4O_2$: C, 73.84; H, 3.61; N, 14.35; Found: C, 73.52; H, 3.71; N, 14.22%.

2.1.3. Formation of N-(4-cyano-1,3-diphenyl-1H-pyrazol-5-yl)acetamide (4)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in dioxane (25 mL), acetyl chloride (1.22 mL, 0.01 mol.) and drops of TEA were added. The mixture was refluxed for 2h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from methanol produced compound **4**.

Yield 79%; deep red crystal; m.p. 96-98 ^{O}C; IR (KBr, *υ*, cm^{-1}) 3288 (NH), 2233 (CN), 1679 (CO), 1606 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.27 (s, 3H; CH_3), 6.75-7.87 (m, 10H; Ar-H), 10.81 (s, 1H, NH; D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 20.4, 117.2, 123.9, 130.7, 133.0, 133.7, 134.2, 140.4, 141.6, 144.5, 150.2 and 159.3; MS: *m/z* 302 (M^+) (4.6%). Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53; Found: C, 71.46; H, 4.55; N, 18.78%.

2.1.4. Formation of N-(4-cyano-1,3-diphenyl-1H-pyrazol-5-yl)benzenesulfonamide (5)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in sodium ethoxide (2 g of Na in 40 mL absolute ethanol), benzene sulphonyl chloride (1.75 g, 0.01 mol.) was added. The mixture was refluxed for 4h.The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from methanol produced compound **5**.

Yield 77%; black crystal; m.p. 106-108 ^{O}C; IR (KBr, *υ*, cm^{-1}) 3201 (NH), 2204 (CN), 1592 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.73-7.88 (m, 15H; Ar-H), 12.17 (s, 1H, NH; D_2O exchangeable); MS: *m/z* 400 (M^+) (26.0%). Anal. Calcd. for $C_{22}H_{16}N_4O_2S$: C, 65.99; H, 4.03; N, 7.99; S, 8.01; Found: C, 66.12; H, 3.96; N, 13.87; S, 8.23%.

2.1.5. Formation of 4,6-diamino-1,3-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (6)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in sodium ethoxide (2 g of Na in 40 mL absolute ethanol), malononitrile (0.66g, 0.01 mol.) was added. The mixture was refluxed for 4h.The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol produced compound **6**.

Yield 80%; black crystal; m.p. 100-102 ^{O}C; IR (KBr, *υ*, cm^{-1}) 3442, 3315, 3290 (NH_2), 2169 (CN), 1633, 1601 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 6.72-7.87 (m, 10H; Ar-H), 10.32 (s, 4H, $2NH_2$; D_2O exchangeable); MS: *m/z* 326 (M^+) (40.9%).Anal. Calcd. for $C_{19}H_{14}N_6$: C, 69.92; H, 4.32; N, 25.75; Found: C, 69.88; H, 4.14; N, 25.98%.

2.1.6. Formation of 4,6-diamino-1,3-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (7)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in sodium ethoxide (2 g of Na in 40 mL absolute ethanol), cyanoacetamide (0.84 g, 0.01 mol.) was added. The mixture was refluxed for 4h.The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol produced compound **7**.

Yield 75%; orange crystal; m.p. 120-122 ^{O}C; IR (KBr, *υ*, cm^{-1}) 3445, 3309, 3281 (NH_2), 1658 (C=O), 1595 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.72-7.86 (m, 10H; Ar-H), 8.89 (s, 4H, $2NH_2$; D_2O exchangeable), 11.24 (s, 2H, $CONH_2$; D_2O-exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 117.1, 123.9, 130.7, 133.0, 133.7, 134.2, 140.9, 141.6, 150.4 and 162.3; MS: *m/z* 344 (M^+) (13.3%). Anal. Calcd. for $C_{19}H_{16}N_6O$: C, 66.27; H, 4.68; N, 24.40; Found: C, 66.05; H, 4.74; N, 24.51%.

2.1.7. Formation of ethyl-4-amino-6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (8)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in ethanol (25 mL), ethyl acetoacetate (1.3 mL, 0.01 mol.) and drops of TEA was added. The mixture was refluxed for 3h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol gave compound **8**.

Yield 83%; beige crystal; m.p. 148-150 ^{O}C; IR (KBr, *υ*, cm^{-1}) 3423, 3311 (NH_2), 1635 (C=O), 1591 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 1.22 (t, J = 6.2 Hz, 3H; $\underline{CH_3}CH_2$), 4.02 (s, 3H; CH_3), 4.20 (q, J = 6.4 Hz, 2H; $CH_3\underline{CH_2}$), 6.72-7.87 (m, 10H; Ar-H), 9.69 (s, 2H, NH_2; D_2O exchangeable). ^{13}C NMR (DMSO-d_6) δ: 18.4, 23.2, 57.2, 117.1, 123.9, 130.7, 133.0, 133.8, 134.2, 140.9, 141.6, 150.4 and 168.3; MS: *m/z* 372 (M^+) (24.2%). Anal. Calcd. for $C_{22}H_{20}N_4O_2$: C, 70.95;

H, 5.41; N, 15.04; Found: C, 70.88; H, 5.23; N, 15.21 %.

2.1.8. Formation of 4-amino-6-oxo-1,3-diphenyl-6,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (9)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in ethanol (25 mL), ethyl cyanoacetate (1.13 mL, 0.01 mol.) and drops of TEA was added. The mixture was refluxed for 3h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol gave compound **9**.

Yield 80%; brown crystal; m.p. 146-148 ^{O}C; IR (KBr, υ, cm^{-1}) 3419, 3312 (NH_2), 2258 (CN), 1746 (C=O), 1631 cm^{-1} (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.72-7.87 (m, 10H; Ar-H), 8.52 (s, 2H, NH_2; D_2O exchangeable), 11.47 (s, 1H, NH; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 117.2, 123.9, 130.7, 133.0, 133.7, 134.2, 141.0, 141.6, 150.5 and 154.1; MS: *m/z* 327 (M^+) (44.3%). Anal. Calcd. for $C_{19}H_{13}N_5O$: C, 69.71; H, 4.00; N, 21.39; Found: C, 69.65; H, 4.14; N, 21.41%.

2.1.9. Formation of 6-Methyl-1,3-diphenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in acetic anhydride (15 mL). The mixture was refluxed for 4h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol gave compound **10**.

Yield 82%; beige crystal; m.p. 100-102 ^{O}C; IR (KBr, υ, cm^{-1}) 3236 (NH), 1681 (C=O), 1608 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.52 (s, 3H; CH_3), 6.72-7.87 (m, 10H; Ar-H), 11.57 (s, 1H, NH; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 24.3, 112.7, 117.5, 123.8, 130.6, 133.1, 133.4, 134.2, 141.0, 141.4 and 167.3; MS: *m/z* 302 (M^+) (9.3%). Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53; Found: C, 71.64; H, 4.59; N, 18.71%.

2.1.10. Formation of 6-methyl-1,3-diphenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-thione (11)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in pyridine (15 mL), phosphorous pentasulphide (4.44 g, 0.01 mol.) was added. The mixture was refluxed for 6h. The reaction mixture after cooling was poured into dil. HCl (100 mL), the solid formed was filtrated, and then recrystallized from petroleum ether 60-80 ^{O}C gave compound **11**.

Yield 76%; black crystal; m.p. 150-152 ^{O}C; IR (KBr, υ, cm^{-1}) 3245 (NH), 1593 (C=N), 1263 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H; CH_3), 6.74-7.86 (m, 10H; Ar-H), 8.62 (s, 1H, NH; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 24.3, 110.6, 117.2, 123.7, 130.3, 133.4, 133.6, 134.2, 141.0, 141.3, 150.6 and 172.6; MS: *m/z* 318 (M^+) (64.2%). Anal. Calcd. for $C_{18}H_{14}N_4S$: C, 67.90; H, 4.43; N, 17.60; S, 10.07; Found: C, 67.78; H, 4.21; N, 17.78; S, 10.23%.

2.1.11. Formation of 4-chloro-6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidine (12)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in phosphorous oxychloride (15 mL). The mixture was refluxed for 3h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from petroleum ether 60-80 ^{O}C gave compound **12**.

Yield 80%; black crystal; m.p. 120-122 ^{O}C; IR (KBr, υ, cm^{-1}) 1622, 1595 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 3H; CH_3), 6.74-7.84 (m, 10H; Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 24.3, 117.2, 123.7, 130.3, 133.0, 133.4, 133.6, 134.2, 141.0, 141.3, 150.7 and 154.5; MS: *m/z* 320 (M^+) (41.45%). Anal. Calcd. for $C_{18}H_{13}N_4Cl$: C, 67.40; H, 4.09; N, 17.47; Cl, 11.05; Found: C, 67.29; H, 4.14; N, 17.43; Cl, 11.14%.

2.1.12. Formation of 2-(6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazine-1-carbothioamide (13)

To a hot solution of compound **12** (3.2 g, 0.01 mol.) in ethanol (25 mL), thiosemicarbazide (0.91 g, 0.01 mol.) was added. The mixture was refluxed for 8h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol produced compound **13**.

Or, to a hot solution of compound **11** (3.18 g, 0.01 mol.) in ethanol (25 mL), thiosemicarbazide (0.91 g, 0.01 mol.) was added. The mixture was refluxed for 8h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol produced compound **13**.

Yield 82%; brown crystal; m.p. 182-184 ^{O}C; IR (KBr, υ, cm^{-1}) 3447, 3357 (NH_2), 3218, 3189 (NH), 1633, 1612 (C=N), 1266 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H; CH_3), 6.74-7.86 (m, 10H; Ar-H), 8.24 (s, 1H, NH; D_2O exchange), 8.87 (s, 1H, NH; D_2O exchangeable), 9.74 (s, 2H, NH_2; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 27.3, 117.1, 129.8, 130.7, 132.3, 133.0, 133.6, 134.1, 134.2, 140.9, 141.5, 150.5 and 177.3; MS: *m/z* 375 (M^+) (14.2%). Anal. Calcd. for $C_{19}H_{17}N_7S$: C, 60.78; H, 4.56; N, 26.11; S, 8.54; Found: C, 60.68; H, 4.47; N, 26.04; S, 8.81%.

2.1.13. Formation of 5-methyl-7,9-diphenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[4,3-c]pyrimidin-3-amine (14)

To a hot solution of compound **12** (3.2 g, 0.01 mol.) in ethanol (25 mL), thiosemicarbazide (0.91 g, 0.01 mol.) was added. The mixture was refluxed for 20h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from acetone produced compound **14**.

Or to a hot solution of compound **13** (3.75 g,

0.01 mol.) in ethanol (25 mL), thiosemicarbazide (0.91 g, 0.01 mol.) was added. The mixture was refluxed for 12h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from acetone produced compound **14**.

Yield 73%; brown crystal; m.p. 268-270 ^{O}C; IR (KBr, υ, cm^{-1}) 3428, 3334 (NH_2), 1634, 1600 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H; CH_3), 6.77-7.84 (m, 10H; Ar-H), 8.61 (s, 2H, NH_2; D_2O exchangeable); MS: *m/z* 341 (M^+) (36.9%). Anal. Calcd. for $C_{19}H_{15}N_7$: C, 66.85; H, 4.43; N, 28.72; Found: C, 66.94; H, 4.37; N, 28.69%.

2.1.14. Formation of 1-(4-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)phenyl)ethan-1-one (15)

To a hot solution of compound **12** (3.2 g, 0.01 mol.) in pyridine (15 mL), p-aminoacetophenone (1.35 g, 0.01 mol.) was added. The mixture was refluxed for 12h. The reaction mixture after cooling was poured into dil. HCl (100 mL), the solid formed was filtrated, and then recrystallized from benzene gave compound **15**.

Yield 83%; brown crystal; m.p. 200-202 ^{O}C; IR (KBr, υ, cm^{-1}) 3200 (NH), 1655 (C=O), 1595 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H; CH_3), 3.13 (s, 3H; $COCH_3$), 6.24 (s, 1H, NH; D_2O exchangeable), 6.74-7.86 (m, 14H; Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 22.8, 26.7, 98.6, 111.1, 118.8, 126.7, 127.0, 128.6, 129.1, 131.9, 135.7, 136.4, 145.2, 152.6, 153.8 and 198.7; MS: *m/z* 419 (M^+) (12.9%). Anal. Calcd. for $C_{26}H_{21}N_5O$: C, 74.44; H, 5.05; N, 16.70; Found: C, 74.28; H, 5.14; N, 16.68%.

2.1.15. Formation of 4-hydrazinyl-6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidine (16)

To a hot solution of compound **12** (3.2 g, 0.01 mol.) in ethanol (25 mL), hydrazine hydrate (0.5 mL, 0.01 mol.) was added. The mixture was refluxed for 6h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol gave compound **16**.

Yield 79 %; brown crystal; m.p. 82-84 ^{O}C; IR (KBr, υ, cm^{-1}) 3419 (NH_2), 3212 (NH), 1594 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H; CH_3), 6.30 (s, 2H, NH_2; D_2O exchangeable), 6.7-7.87 (m, 10H; Ar-H), 7.94 (s, 1H, NH; D_2O exchangeable); MS: *m/z* 316 (M^+) (20.1%). Anal. Calcd. for $C_{18}H_{16}N_6$: C, 68.34; H, 5.10; N, 26.56; Found: C, 68.15; H, 5.14; N, 26.71%.

2.1.16. Formation of ethyl 2-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)acetate (17)

To a hot solution of compound **10** (3.02 g, 0.01 mol.) in acetonitrile (25 mL), ethyl chloroacetate (1.22 mL, 0.01 mol.) and potassium carbonate (1.38 g, 0.01 mol.) were added. The mixture was refluxed for 12h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol gave compound **17**.

Yield 77%; beige crystal; m.p. 110-112 ^{O}C; IR (KBr, υ, cm^{-1}) 1680 (C=O), 1606, 1572 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 1.28 (t, J = 6.6 Hz, 3H; $\underline{CH_3}CH_2$), 2.50 (s, 3H; CH_3), 4.17 (q, J = 7.2 Hz, 2H; $CH_3\underline{CH_2}$), 4.52 (s, 2H; O$\underline{CH_2}$CO), 6.75-7.84 (m, 10H; Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 15.6, 22., 56.9, 66.3, 126.9, 128.7, 129.1, 129.3, 129.8, 130.1, 134.1, 135.9, 140.7 and 171.4; MS: *m/z* 388 (M^+) (26.3%); Anal. Calcd. for $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42; Found: C, 67.96; H, 5.01; N, 14.68%.

2.1.17. Formation of 2-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)acetohydrazide (18)

To a hot solution of compound **17** (3.88 g, 0.01 mol.) in ethanol (25 mL), hydrazine hydrate (0.5 mL, 0.01 mol.) was added. The mixture was refluxed for 6h. The reaction mixture after cooling was poured into cold ice water, the solid formed was filtrated, and then recrystallized from acetic acid gave compound **18**.

Yield 75%; yellow crystal; m.p. 188-190^{O}C; IR (KBr, υ, cm^{-1}) 3424 (NH_2), 3273 (NH), 1661 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.51 (s, 3H; CH_3), 4.27 (s, 2H; O$\underline{CH_2}$CO), 6.39 (s, 1H, NH; D_2O exchangeable); 6.76-7.86 (m, 10H; Ar-H), 9.28 (s, 2H, NH_2; D_2O exchangeable); MS: *m/z* 374 (M^+) (25.6%). Anal. Calcd. for $C_{20}H_{18}N_6O_2$: C, 64.16; H, 4.85; N, 22.45; Found: C, 64.02; H, 4.74; N, 22.68%.

2.1.18. Formation of 2-(2-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)acetyl)hydrazine-1-carbodithioic acid (19)

To a hot solution of compound **18** (3.74 g, 0.01 mol.) in pyridine (15 mL), carbon disulphide (5mL) was added. The mixture was refluxed for 6h. The reaction mixture after cooling was poured into dil. HCl (100 mL), the solid formed was filtrated, and then recrystallized from ethanol gave compound **19**.

Yield 79%; brown crystal; m.p. 106-108 ^{O}C; IR (KBr, υ, cm^{-1}) 3440 (NH), 2564 (SH), 1658 (C=O), 1601, 1592 (C=N), 1259 (C=S); ^{1}H-NMR (DMSO-d_6) δ: 1.95 (s, 1H, SH; D_2O exchangeable), 2.50 (s, 3H; CH_3), 4.13 (s, 2H; O$\underline{CH_2}$CO), 6.68-7.87 (m, 10H; Ar-H), 9.62 (s, 1H, NH; D_2O exchangeable), 10.32 (s, 1H, NH; D_2O exchangeable); MS: *m/z* 450 (M^+) (5.3%). Anal. Calcd. for $C_{21}H_{18}N_6O_2S_2$: C, 55.98; H, 4.03; N, 18.65; S, 14.23; Found: C, 55.76; H, 3.95; N, 18.88; S, 14.41%.

2.1.19. Formation of 5-(((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)-1,3,4-oxadiazole-2-thiol (20)

To a hot solution of compound **19** (4.5 g, 0.01 mol.) in ethanol (25 mL), few drops of piperidine was added. The mixture was refluxed for 6h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol gave compound **20**.

Yield 81%; red crystal; m.p. 120-122 ^{O}C; IR (KBr, υ, cm^{-1}) 2529 (SH), 1599, 1591 (C=N). ^{1}H-NMR (DMSO-d$_6$) δ: 2.51 (s, 3H; CH$_3$), 3.78 (s, 1H, SH; D$_2$O exchangeable), 4.14 (s, 2H; OCH$_2$CO), 6.72-7.84 (m, 10H; Ar-H); ^{13}C-NMR (DMSO-d$_6$) δ: 25.9, 69.7, 112.0, 118.7, 125.6, 126.9, 127.3, 127.9, 128.3, 128.9, 129.1, 129.3, 129.5, 130.1, 136.411 and 145.4; MS: *m/z* 416 (M$^+$) (31.6%). Anal. Calcd. for $C_{21}H_{16}N_6O_2S$: C, 60.57; H, 3.87; N, 20.18; S, 7.70; Found: C, 60.42; H, 3.91; N, 20.06; S, 7.81%.

2.1.20. Formation of 2-hydrazinyl-5-(((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)-1,3,4-oxadiazole (21)

To a hot solution of compound **20** (4.16 g, 0.01 mol.) in ethanol (25 mL), hydrazine hydrate (0.5 mL, 0.01 mol.) was added. The mixture was refluxed for 4h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from benzene gave compound **21**.

Yield 76%; beige crystal; m.p. 280-282 ^{O}C; IR (KBr, υ, cm^{-1}) 3347, 3300 (NH$_2$), 3225 (NH), 1599, 1591 (C=N); ^{1}H-NMR (DMSO-d$_6$) δ: 2.50 (s, 3H; CH$_3$), 4.19 (s, 2H; OCH$_2$CO); 6.72-7.86 (m, 10H; Ar-H), 8.72 (s, 1H, NH: D$_2$O exchangeable), 10.29 (s, 2H, NH$_2$; D$_2$O exchangeable); MS: *m/z* 414 (M$^+$) (15.0%). Anal. Calcd. for $C_{21}H_{18}N_8O_2$: C, 60.86; H, 4.38; N, 27.04; Found: C, 60.91; H, 4.41; N, 26.89%.

2.1.21. Formation of 5-(((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)-1H-1,2,4-triazole-3-thiol (22)

To a hot solution of compound **17** (3.88 g, 0.01 mol.) in ethanol (25 mL), thiosemicarbazide (0.91 g, 0.01 mol.) and sodium hydroxide (1.5 gm) were added. The mixture was refluxed for 6h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from ethanol gave compound **22**.

Yield 81%; golden yellow crystal; m.p. 140-142 ^{O}C; IR (KBr, υ, cm^{-1}) 3245 (NH), 2529 (SH), 1599, 1591 (C=N). ^{1} H-NMR (DMSO-d$_6$) δ: 2.50 (s, 3H; CH$_3$), 4.21 (s, 2H; OCH$_2$), 6.72-7.86 (m, 10H; Ar-H), 12.84 (s, 1H, SH; D$_2$O exchangeable), 13.33 (s, 1H, NH; D$_2$O exchangeable); MS: *m/z* 415 (M$^+$) (31.6%). Anal. Calcd. for $C_{21}H_{17}N_7OS$: C, 60.71; H, 4.12; N, 23.60; S, 7.72; Found: C, 60.66; H, 4.04; N, 23.77; S, 7.89%.

2.1.22. Formation of (4-cyano-1,3-diphenyl-1H-pyrazol-5-yl)glycine (23)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in methanol (25 mL), chloro acetic acid (0.95 g, 0.01 mol.). The mixture was refluxed for 4h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from methanol gave compound **23**.

Yield 75%; brown crystal; m.p. 146-148 ^{O}C; IR (KBr, υ, cm^{-1}) 3441 (OH), 3211 (NH), 2203 (CN), 1731 (C=O), 1591 (C=N); ^{1}H-NMR (DMSO-d$_6$) δ: 4.27 (s, 2H; NHCH$_2$CO), 6.72-7.87 (m, 10H; Ar-H), 9.53 (s, 1H, NH; D$_2$O exchangeable), 12.57 (s, 1H, OH; D$_2$O exchangeable); MS: *m/z* 318 (M$^+$) (22.2%) . Anal. Calcd. for $C_{18}H_{14}N_4O_2$: C, 67.92; H, 4.43; N, 17.60; Found: C, 67.78; H, 4.57; N, 17.62%.

2.1.23. Formation of 4-amino-1,3-diphenyl-1,6-dihydropyrrolo[2,3-c]pyrazole-5-carboxylic acid (24)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in sodium ethoxide (2 g of Na in 40 mL absolute ethanol), chloro acetic acid (0.95 g, 0.01 mol.) was added. The mixture was refluxed for 3h.The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol produced compound **24**.

Yield 78%; red crystal; m.p. 170-172 ^{O}C; IR (KBr, υ, cm^{-1}) 3476 (OH), 3414, 3311 (NH$_2$), 3223 (NH), 1709 (C=O), 1645, 1593 (C=N); ^{1}H-NMR (DMSO-d$_6$) δ: 5.08 (s, 2H, NH$_2$; D$_2$O exchangeable), 6.72-7.89 (m, 10H; Ar-H), 10.0 (s, 1H, NH; D$_2$O exchangeable), 10.40 (s, 1H, OH; D$_2$O exchange); MS: *m/z* 318 (M$^+$) (9.8%). Anal. Calcd. for $C_{18}H_{14}N_4O_2$: C, 67.92; H, 4.43; N, 17.60; Found: C, 67.81; H, 4.44; N, 17.51%.

2.1.24. Formation of 4,6-diphenyl-1,6-dihydropyrazolo[3,4-c]pyrazol-3-amine (25)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in ethanol (25 mL), hydrazine hydrate (0.5 g, 0.01 mol.) was added. The mixture was refluxed for 3h.The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from methanol produced compound **25**.

Yield 76%; brown crystal; m.p. 140-142 ^{O}C; IR (KBr, υ, cm^{-1}) 3385, 3313 (NH$_2$), 3201 (NH), 1627, 1592 (C=N); ^{1}H-NMR (DMSO-d$_6$) δ: 6.73-7.87 (m, 10H; Ar-H), 8.68 (s, 1H, NH; D$_2$O exchangeable), 10.31 (s, 2H, NH$_2$; D$_2$O exchangeable); MS: *m/z* 275 (M$^+$) (8.1%). Anal. Calcd. for $C_{16}H_{13}N_5$: C, 69.80; H, 4.76; N, 25.44; Found: C, 69.67; H, 4.88; N, 25.45%.

2.1.25. Formation of 4-amino-1,3-diphenyl-1,7-dihydro-6H-pyrazolo[3,4-d]pyrimidin-6-one (26)

To a hot solution of enaminonitrile **1** (2.6 g,

0.01 mol.) in acetic acid (15 mL), urea (0.6 g, 0.01 mol.) and with two drops of HCl were added. The mixture was refluxed for 3h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from toluene afforded compound **26**.

Yield 76%; red crystal; m.p. 106-108 ^{O}C; IR (KBr, υ, cm^{-1}) 3456, 3312 (NH_2, NH), 1688 (C=O), 1611 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.69-7.92 (m, 10H; Ar-H), 9.55 (s, 2H, NH_2; D_2O exchangeable), 10.28 (s, 1H, NH; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 117.1, 123.3, 130.2, 133.6, 134.1, 134.2, 140.9, 141.6, 150.4 and 159.8; MS: *m/z* 303 (M^+) (37.7%). Anal. Calcd. for $C_{17}H_{13}N_5O$: C, 67.32; H, 4.32; N, 23.09; Found: C, 67.14; H, 4.39; N, 23.44%.

2.1.26. Formation of 4-amino-1,3-diphenyl-1,7-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione (27)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in sodium ethoxide (2 g of Na in 40 mL absolute ethanol), thiourea (0.76 g, 0.01 mol.) was added. The mixture was refluxed for 3h.The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from toluene produced compound **27**.

Yield 75%; red crystal; m.p. 220-222 ^{O}C; IR (KBr, υ, cm^{-1}) 3397, 3311 (NH_2), 3200 (NH), 1600, 1593 (C=N), 1287 (C=S); ^{1}H-NMR (DMSO-d_6) δ: 6.72-7.87 (m, 10H, Ar-H), 10.33 (s, 2H, NH_2, D_2O exchangeable), 13.14 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 117.2, 123.9, 130.7, 133.0, 133.8, 134.1, 134.2, 140.9, 141.6, 150.5 and 189.1; MS: *m/z* 319 (M^+) (10.7%). Anal. Calcd. for $C_{17}H_{13}N_5S$: C, 63.93; H, 4.10; N, 21.93; S, 10.04; Found: C, 63.74; H, 4.22; N, 22.04; S, 10.00%.

2.1.27. Formation of 4-ethoxy-1,3,6-triphenyl-1H-pyrazolo[3,4-d]pyrimidine (28)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in ethanol (25 mL), benzaldehyde (1.09 mL, 0.01 mol.) and with sodium hydroxide (2 gm) were added. The mixture was refluxed for 4h.The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from ethanol produced compound **28**.

Yield 81%; brown crystal; m.p. 138-140 ^{O}C; IR (KBr, υ, cm^{-1}) 1633, 1600 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 1.31 (t, J = 6.0 Hz, 3H; $\underline{CH_3}CH_2$), 4.20 (q, J = 6.6 Hz, 2H; $CH_3\underline{CH_2}$), 6.72-7.86 (m, 15H; Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 15.9, 56.8, 111.9, 118.8, 125.6, 127.9, 127.9, 135.7, 136.6 and 145.2; MS: *m/z* 392 (M^+) (14.1%). Anal. Calcd. for $C_{25}H_{20}N_4O$: C, 76.51; H, 5.14; N, 14.28; Found: C, 76.43; H, 5.28; N, 14.01%.

2.1.28. Formation of 6-(5-amino-1,3-diphenyl-1H-pyrazol-4-yl)-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (29)

To a hot solution of enaminonitrile **1** (2.6 g, 0.01 mol.) in ethanol (25 mL), two drops of TEA was added. The mixture was refluxed for 7h.The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from methanol produced compound **29**.

Yield 85%; red crystal; m.p. 160-162 ^{O}C; IR (KBr, υ, cm^{-1}) 3427, 3311 (NH_2), 1591 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 6.72-7.86 (m, 20H; Ar-H), 9.41 (s, 2H, NH_2; D_2O exchangeable), 10.57 (s, 2H, NH_2; D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 112.0, 118.7, 125.6, 127.9, 128.6, 129.1, 135.9, 136.4 and 145.3; MS: *m/z* 520 (M^+) (24.7%). Anal. Calcd. for $C_{32}H_{24}N_8$: C, 73.83; H, 4.65; N, 21.52; Found: C, 73.77; H, 4.72; N, 21.51%.

2.2. Biological assay:

2.2.1. Antimicrobial activity:

The Sensitiveness Tests were carried out according to "National Committee for clinical laboratory Standards 1993" NCCLS recommendations. Screening tests concerning the zone of inhibition were accomplished by using the well diffusion method. The inoculum suspension was synthesized from colonies grown over-night on an inoculated and agar plate onto Mueller Honton broth "fungi using the malt broth". Sterile swabs were immersed in the crossing and used to vaccinate. Mueller-Honton agar plates fungi using malt agar plates. The pyrazole derivatives were dissolved in Sigma-Aldrich dimethyl sulfoxide "DMSO" with different concentrations "50 mg/ml". The inhibition zones were evaluated around each well at 37^{O}C after 24h "for fungi after 48h" controls using Sigma-Aldrich DMSO were adequately done [16-17].

2.2.2. Transmission Electron Microscopy:

The samples of the TEM were fixed in 3% 1,5-pentanedia,l rinsed in phosphate as a buffer solution, and post fixed in the solution of the potassium permanganate at room temperature for 5 min. The samples were diluted in a series of ethanol ranging from 90% to 10% for 15 min in each alcohol and finally for 30 min with absolute ethanol. Samples were filtered with acetone and epoxy resin via a graded series until finally in the pure resin Ultra-thin section was collected at copper grids. The sections were then double-stained in uranyl acetate followed by lead citrate. Stained sections were evaluated with a JEOL - JEM 1010 transmission electron microscope at 70 kV at "The Regional Center for Mycology and Biotechnology", Al- Azhar University [18-19].

3. Results and discussion:

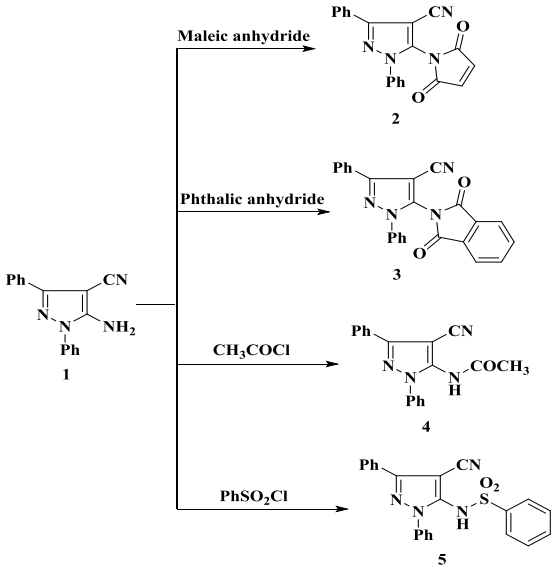
3.1. Chemistry:

The new pyrazoles were prepared following the reaction sequences depicted in Scheme 1-5. The starting enaminonitrile **1** was prepared by literature known procedure [14] using malononitrile, phenyl hydrazine, and benzaldehyde in a one-pot reaction.

Compound **1** was condensed with maleic anhydride and phthalic anhydride in glacial acetic acid afforded 2,5-dioxopyrole derivative **2** and 1,3-dioxoisoindoline derivative **3**. The structure of **2** and **3** was supported by the appearance of bands of C=O group and the disappearance of υNH_2 group.

Acetyl derivative **4** was produced from the treatment of compound **1** with acetyl chloride via elimination of one HCl molecule, however, its IR spectrum revealed strong bands at 2233 and 1679 cm^{-1} which are specific for the CN and C=O groups, its ^{1}H-NMR spectrum revealed bands at 2.27 and 10.81 ppm corresponding to CH_3 and NH protons, respectively. Its ^{13}C-NMR spectrum revealed signals at 20.4 and 159.3 ppm corresponding to CH_3 and C=O, respectively. Its molecular ion peak appeared at m/z 302 (M^+) in the mass spectrum which corresponding to $C_{18}H_{14}N_4O$.

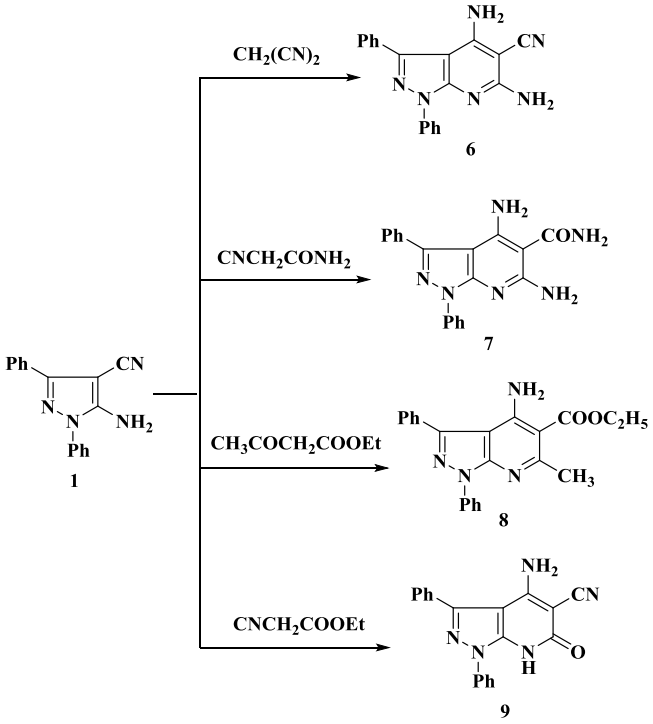
Similarly, compound **1** was reacted with benzene sulfonyl chloride gave the benzenesulfonamide derivative **5**. (Scheme 1).



Scheme 1. Synthetic methods for Amide and Sulfonamide derivatives 2–5.

The bifunctional starting material **1** can use to synthesize the pyrazolopyridine derivatives **6-9** via its reaction with different active methylene compounds, namely malononitrile, cyano acetamide, ethyl acetoacetate, ethyl cyanoacetate in the presence of a catalytic base (EtONa or Et_3N). Different data and elemental analysis showed excellent consistent for the prepared structures. IR of compound **6** revealed bands at 3442, 3315, 3290 cm^{-1} characteristic for the NH_2 group. Its ^{1}H-NMR spectrum showed a band corresponding to NH_2 at 10.32 ppm. Its mass spectrum revealed a molecular ion peak at 326 (M^+) corresponding to $C_{19}H_{14}N_6$.

However, compound **9** IR spectrum revealed bands for the NH_2 at 3419, 3312 $cm^{-1}$and C=O at 1746 cm^{-1}. Its ^{1}H-NMR spectrum revealed bands corresponding to NH_2 and NH at 8.52 and 11.47 ppm, respectively. Its ^{13}C-NMR spectrum appeared to signal at 154.1 corresponding to C=O. Its mass spectrum revealed a molecular ion peak at 327 (M^+) corresponding to $C_{19}H_{13}N_5O$. (Scheme 2)



Scheme 2. Synthetic methods for Pyrazolopyrimidine derivatives 6–9.

Heating compound **1** with acetic anhydride gave the pyrazolopyrimidine-4-one derivative **10**. The reaction probably proceeds via the hydrolysis of the CN group to $CONH_2$ group, then acetylation of the amino group, followed by closure and elimination of one molecule of water. Compound structure **10** was confirmed by using spectroscopic data and also its reaction with phosphorous pentasulphide and phosphorous oxychloride to give the pyrazolopyrimidine thione **11** and chloropyrazolopyrimidine **12**, respectively. Their IR spectrum was revealed devoid of $\upsilon C=O$ group.

The product of the reaction of compound **12** with thiosemicarbazide was found to depend upon the time of the reaction. When the reaction was carried out for 8h, the corresponding hydrazine carbothiamide **13** was obtained, however, when the reaction was carried out for 20h the Pyrazolotriazolopyrimidine **14** was obtained. Interestingly, heating compound **13** for an additional

12h, compound **14** was obtained, through cyclization and elimination of one H_2S molecule.

Compound **13** structure was confirmed by spectroscopic data. Its IR spectra revealed bands at 3447, 3357, 3218, 3189 corresponding to NH_2 and 2NH groups. Its ^{1}H-NMR spectrum showed bands corresponding to 2NH and NH_2 at 8.24, 8.87, and 9.74 ppm, respectively, while in its ^{13}C-NMR spectrum appeared signals at 2.73, and 177.3 corresponding to CH_3 and C=S, respectively.

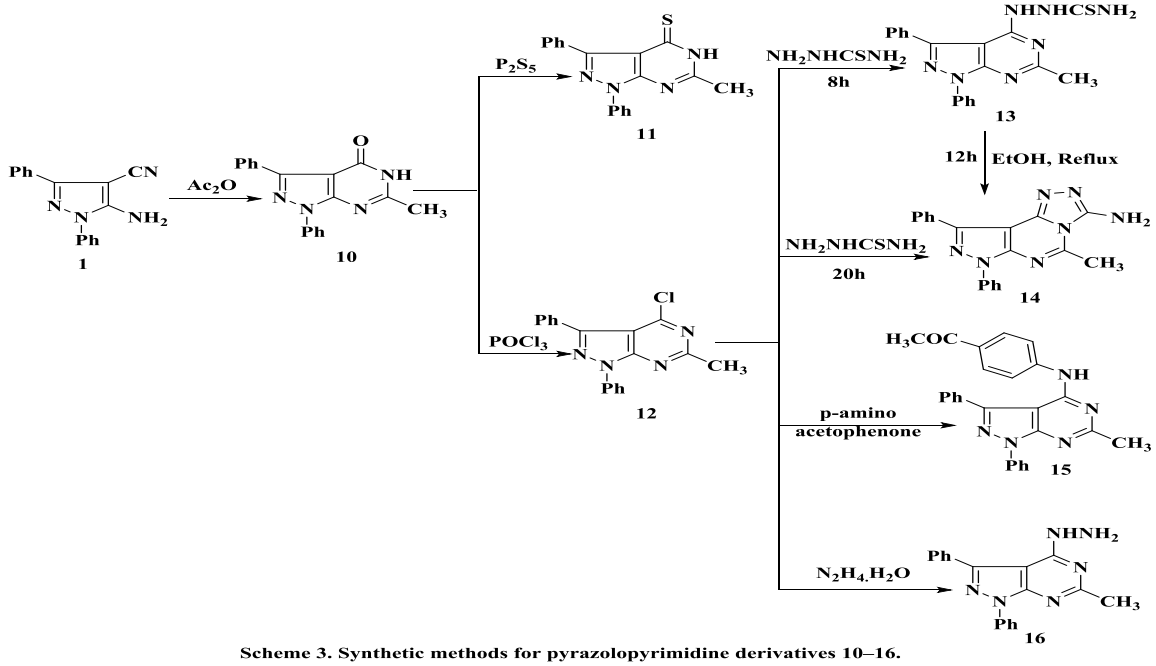
On the other hand, the reaction of 12 with 4-aminoacetophenone and hydrazine hydrate afforded compounds 15 and 16, respectively, through the elimination of one HCl molecule. (Scheme 3).

1,3,4-Oxadiazole derivative **21** was synthesized through consecutive reactions starting from pyrazolopyrimdine-4-one derivative **10**. Thus, the reaction of compound **10** with ethyl chloroacetate gave the oxyethylacetate derivative **17**, which treated with hydrazine hydrate producing hydrazide derivative **18**, which on reaction with carbon disulphide afforded hydrazine-1-carbodithioic acid derivative **19**, which undergo cyclization by refluxing in ethanol with few drops of piperidine giving 1,3,4-oxadiazole-2-thiol derivative **20**, which finally reacted with hydrazine hydrate forming 1,3,4-oxadiazole derivative **21**.

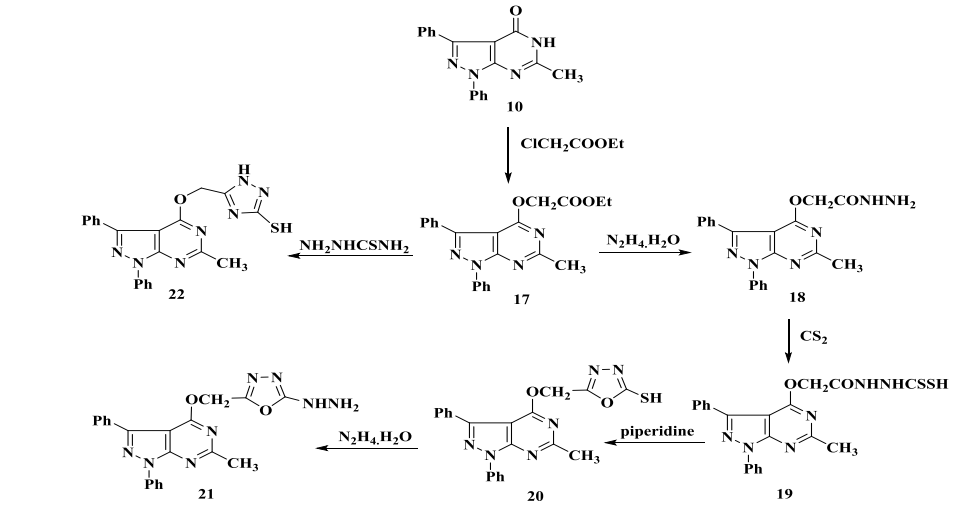
The structure of desired compounds was illustrated by using different spectroscopic tools. For example, IR spectrum of oxadiazole derivative **21** revealed bands at 3347, 3300 (NH_2), 3225 (NH), 2529 (SH), 1599, 1591 cm^{-1} (C=N). It ^{1}H-NMR spectrum exposed bands corresponding to OCH_2CO, NH, and NH_2 proton at 4.19, 8.72, and 10.29 ppm, respectively. Its molecular ion peak appeared at m/z=414 [M^+] in mass spectrum which corresponding to $C_{21}H_{18}N_8O_2$.

On the other hand, compound **17** treated with thiosemicarbazide gave 1,2,4-triazole-3-thiol **22** derivative. Different spectral data tools and elemental analysis showed excellent consistent for the proposed structure. Its IR spectrum revealed bands at 3245 (NH), 2529 (SH), 1599, 1591 cm^{-1} (C=N). Its ^{1}H-NMR spectrum exposed bands corresponding to OCH_2, SH, and NH proton at 4.21, 12.84, and 13.33 ppm, respectively. Its molecular ion peak appeared at m/z=415 [M^+] in the mass spectrum which corresponding to $C_{21}H_{17}N_7OS$. (Scheme 4).

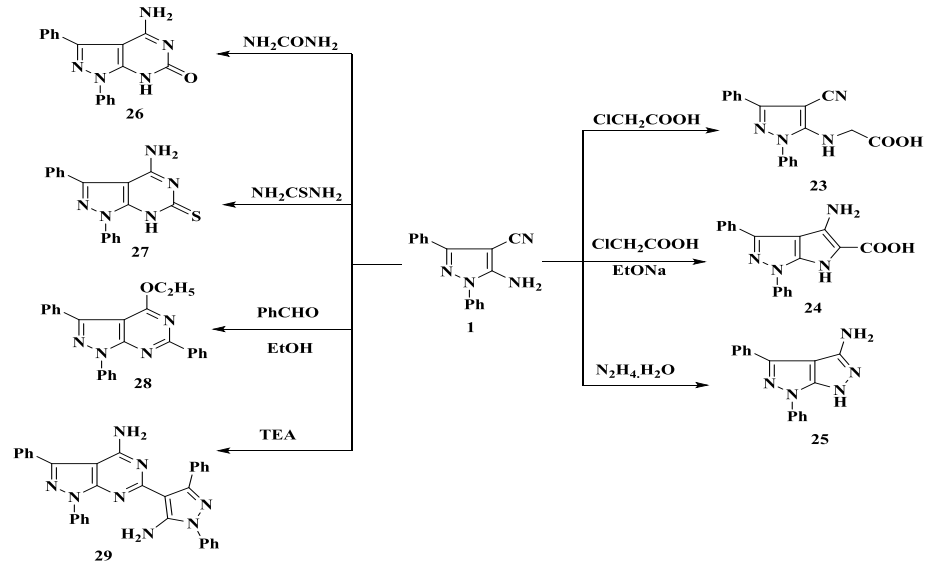
Finally, (Scheme 5) the showed synthesis of bioactive heterocycle derivatives via reaction of enaminonitrile **1** with different electrophilic and nucleophilic reagents. Refluxing enaminonitrile **1** with mono chloro acetic acid depend upon the reaction conditions. Thus, the reaction of compound **1** with mono chloro acetic acid in methanol gave glycine derivative **23**, while, when refluxing the reaction mixture in sodium ethoxide afforded pyrrolopyrazole derivative **24**. Also, enaminonitrile **1** was used as akey intermediate in interesting bicyclic compounds synthesis, via its reactions with hydrazine hydrate, urea, thiourea, benzaldehyde, and TEA, to afford pyrazolopyrazol-3-amine **25**, pyrazolopyrimidin-6-one **26**, pyrazolopyrimidine-6-thione **27**, pyrazolopyrimidine **28** and pyrazolopyrimidin-4-amine **29**, respectively. The newly prepared compounds structures were established by using, IR, ^{1}H-NMR, ^{13}C-NMR, M.S, and elemental analysis



Scheme 3. Synthetic methods for pyrazolopyrimidine derivatives 10–16.



Scheme 4. Synthetic methods for pyrazolopyrimidine derivatives 17–22.



Scheme 5. Synthetic methods for pyrazole derivatives 23–29.

Antimicrobial activity:

The antimicrobial examination and transmission electron microscopy of the tested prepared pyrazole derivatives were accomplished at the Regional Center for Biotechnology and Mycology, Al-Azhar University, Nasr City, Egypt. The newly twenty-eight prepared target derivatives were evaluated in vitro for antimicrobial activities versus “Staphylococcus aureus” “RCMB 010010” and “Bacillis subtilis” “RCMB 015 (1)” as Gram positive bacteria example and “Escherichia coli” “RCMB 010052” and “proteus vulgaris” “RCMB 004 (1)” as Gram negative bacteria example. Also evaluated the prepared compounds in vitro for potential antifungal activities versus “Aspergillus fumigates” "RCMB 002008" and “Candida albicans” "RCMB 005003". The antibacterial and antifungal activities preliminary screening was determinate by using the agar diffusion method. The used reference drugs are Ketoconazole and Gentamycin. The tested compounds results were recorded as inhibition zones average diameter (IZ) of bacterial and/or fungal which growth in mm around the discs. The zone diameters of inhibition referred to the preliminary test concentration test (5 mg/mL) which is shown in Table 1 and Figures 1-6. Transmission Electron Microscopy TEM is one of the important techniques which produce images of biological specimens with high resolution. It is used to investigate the mode of action of the prepared pyrazole derivatives of the highest antifungal and antibacterial activities on the structure of bacterial and fungi The TEM images high resolution obtained from the used package of electrons "which have very short wavelengths" as the illuminating radiation source. The action mode referred to preliminary compounds which imaging by TEM against bacterial and fungi shown in Figure 7.

Table (1): Antimicrobial Activity as mean zones of inhibition (mm) against some microorganisms:

Sample Code	Tested microorganisms					
	Fungi		G +ve bacteria		G –ve bacteria	
	A. fumigatus	***C. albicans***	***S. aureus***	***B. subtilis***	***E.coli***	***P.vulgaris***
Ketoconazole	17	20	NT	NT	NT	NT
Gentamycin	NT	NT	24	26	30	25
1	15	16	11	NA	15	18
2	14	18	16	10	15	16
3	20	22	NA	19	10	20
4	15	13	NA	14	NA	20
5	9	14	NA	10	11	18
6	NA	15	NA	9	8	NA
7	8	15	NA	13	10	17
8	NA	10	NA	NA	9	15
9	NA	8	NA	NA	11	14
10	13	12	NA	10	NA	16
11	18	15	NA	19	NA	24
12	16	NA	NA	12	NA	12
13	9	12	8	8	12	NA
14	NA	NA	NA	8	NA	15
15	NA	26	NA	8	NA	18
16	10	NA	NA	NA	NA	12
17	24	25	15	15	12	20
18	17	20	16	19	17	21
19	16	18	12	14	12	15
20	NA	27	NA	14	NA	14
21	NA	18	NA	10	NA	13
22	13	14	11	12	15	16
23	12	13	10	14	14	20
24	NA	18	NA	17	13	16
25	10	NA	12	8	9	13
26	NA	14	NA	8	NA	13
27	NA	15	NA	11	NA	13
28	NA	19	NA	14	NA	12
29	NA	19	NA	14	NA	12

* NA: No. Effect.
* NT: Not Tested.

In Vitro Antibacterial activity:

All the tested pyrazole derivatives results showed variable antibacterial activities against the Gram positive and Gram negative bacteria. Preliminary antibacterial screening was carried out for the pyrazole derivatives **2-9**. According to these derivatives series **3** and **4** showed good inhibitory activities versus the tested p. vulgaris bacteria as present in Figure 1. Compound **2** showed good result by comparison with Gentamycin as shown from their inhibitory antibacterial activity versus the screened E. coli, while, derivatives **3**, **5**, **6**, **7**, **8** and **9** exhibit 50% less activity and compound **4** has no effect compared to gentamycin against E. coli. The results illustrated in Figure 1 detect that the derivatives **3**, **4** and **7** represent strong antibacterial activity against the gram positive bacteria B. subtilis growth. While, only compound **2** showed strong antibacterial activity versus S. aureus growth.

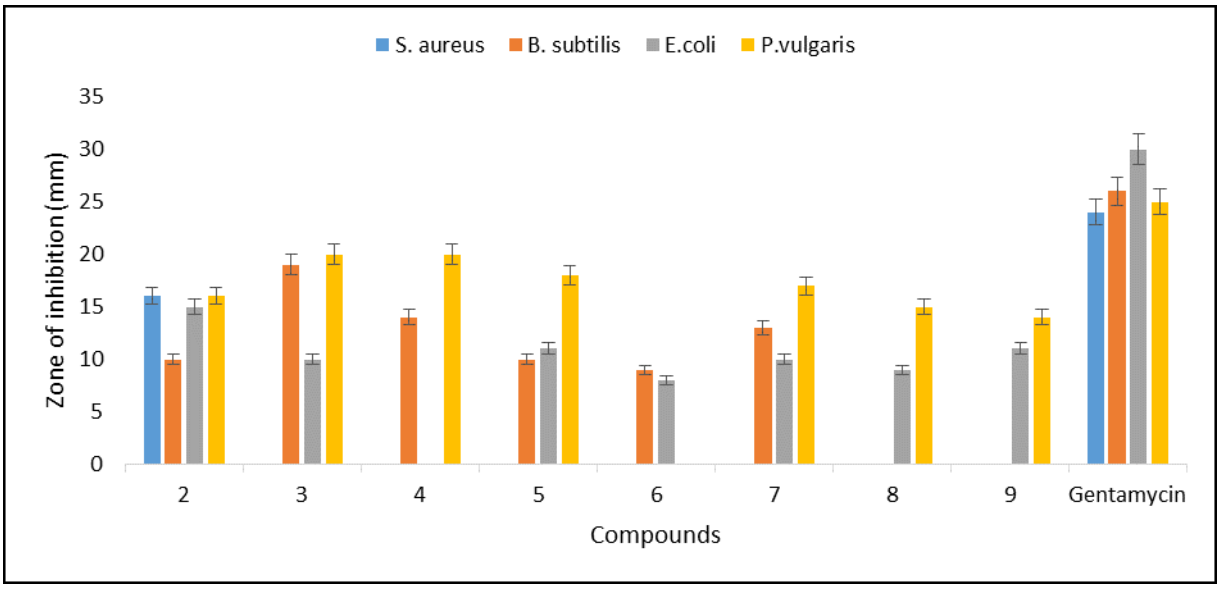


Figure 1. Pyrazole derivatives **2-9** preliminary antibacterial activity versus Gram positive and negative bacteria.

By investigating the compounds **10-16** biological activity as antibacterial agents as shown in Figure 2. According to this series derivatives **12** and **16** showed very good inhibitory antibacterial activity versus the screened P. vulgaris bacteria. While, compounds **15** and **11** exhibit moderated activity and compounds **14** and has no effect compared to gentamycin against P. vulgaris. While, only compound **2** revealed strong antibacterial activity against E. coil. While, it was revealed that compounds **10-16** were active with wide spectrum activity against B. subtilis as anti-gram positive bacterial agents. Unfortunately, all the prepared compounds were inactive versus S. aureus.

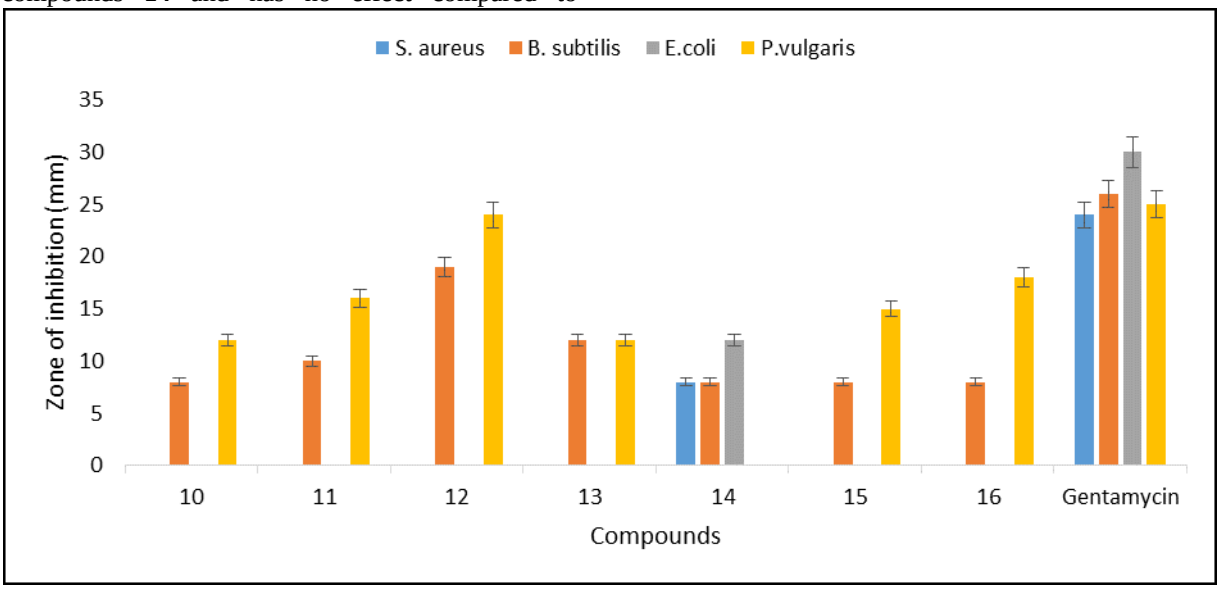


Figure 2. Pyrazole derivatives **10-16** preliminary antibacterial activity versus Gram positive and negative bacteria.

By investigating the compounds **17-22** biological activity as antibacterial agents as shown in Figure 3. According to this series derivatives **18** and **19** showed very good inhibitory antibacterial activity versus the screened *P. vulgaris* bacteria. While, compounds **17**, **20**, **21** and **22** exhibit moderated activity compared to gentamycin against *P. vulgaris*. While, only compound **19** revealed moderated antibacterial activity against *E. coil*. While, compounds **17**, **20**, **21** and **22** exhibit moderated activity compared to gentamycin against *E. coil*. While, it was revealed that compounds **18-22** were active with wide spectrum activity against *B. subtilis* as anti-gram positive bacterial agents. Unfortunately, compounds **17** has no effect compared to gentamycin against *B. subtilis*. While, compounds **18**, **19** and **20** showed moderated antibacterial activity against *S. aureus* growth.

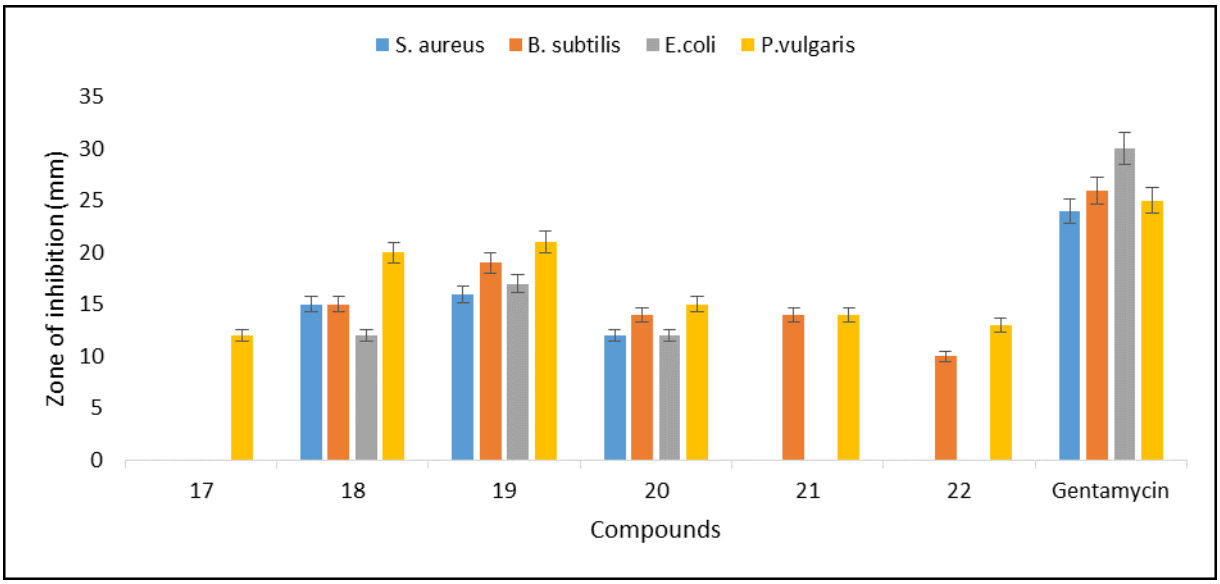


Figure 3. Pyrazole derivatives **17-22** preliminary antibacterial activity versus Gram positive and negative bacteria.

By investigating the compounds **23-29** biological activity as anti-bacterial agents as shown in Figure 4. According to this series compounds **22-29** were active with wide spectrum activity against *P. vulgaris* bacteria as anti-gram negative bacterial agents. While, derivative **26** showed weak inhibitory activity versus only the screened *E. coli* bacteria. While, derivatives **23-29** showed very broad spectrum inhibitory activity versus against *B. subtilis* and *S. aureus*.

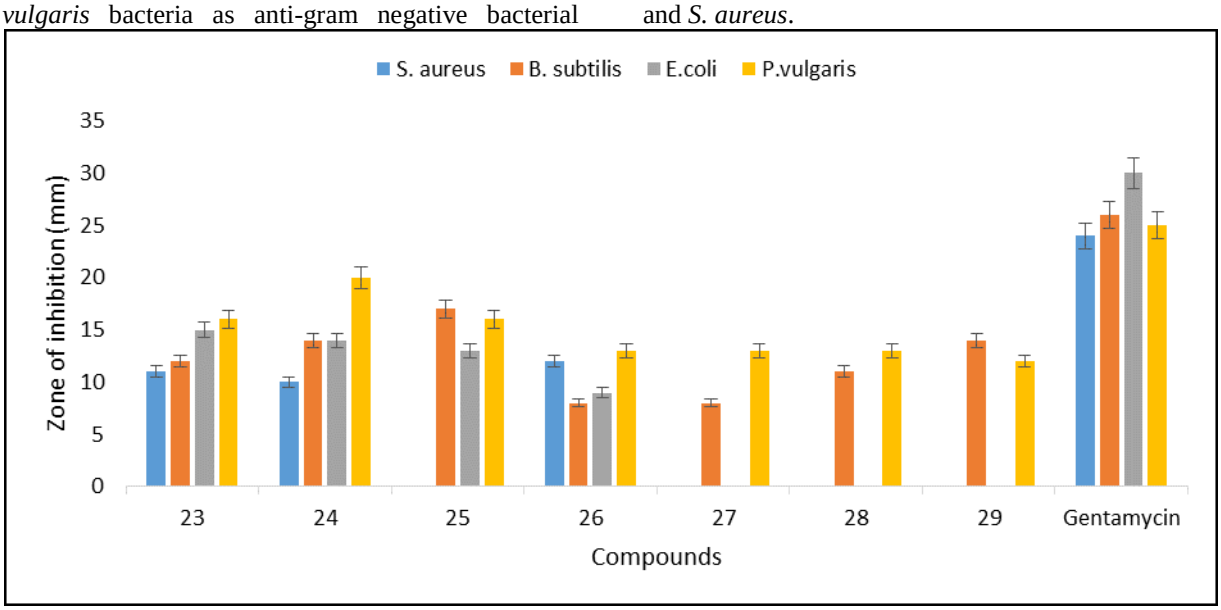


Figure 4. Pyrazole derivatives **23-29** preliminary antibacterial activity versus Gram positive and negative bacteria.

In Vitro Antifungal activity:

By investigating the compounds **2-16** biological activity as antibacterial agents as shown in Figure 5. According to this series compounds **2-16** were active with wide spectrum activity against *A. fumigatus* and *C. albicans* as antifungal agents. Interestingly, the compound **3** was displayed antifungal activity more than ketoconazole against *C. albicans* and *A. fumigatus* which reach to 110% and 117%, respectively. Also, compound **12** was displayed 105% antifungal activity by comparison with ketoconazole against *A. fumigatus*. Moreover, compounds **13**, **10**, **4** and **2** were displayed 94%, 88%, 88%, 82% antifungal activity by comparison with ketoconazole against *A. fumigatus* as shown in Figure 5. While, compounds **7**, **5**, **14** and **11** were displayed 47%, 53%, 53% and 76% antifungal activity by comparison with ketoconazole against *A.*

fumigatus. Unfortunately, compounds **6**, **8**, **9**, **15** and **16** were inactive against *A. fumigatus*. On the other hand, the compound **16** was displayed antifungal activity more than ketoconazole against *C. albicans* which reach to 190%. Moreover, compounds **2**, **6**, **7** and **12** were displayed 90%, 75%, 75%, 75% antifungal activity by comparison with ketoconazole against *C. albicans*. While, compounds **5**, **4**, **11**, **14**, **8** and **9** were displayed 70%, 65%, 60%, 60%, 50% and 40% antifungal activity by comparison with ketoconazole against *A. fumigatus*. Unfortunately, compound **10** was inactive against *C. albicans*.

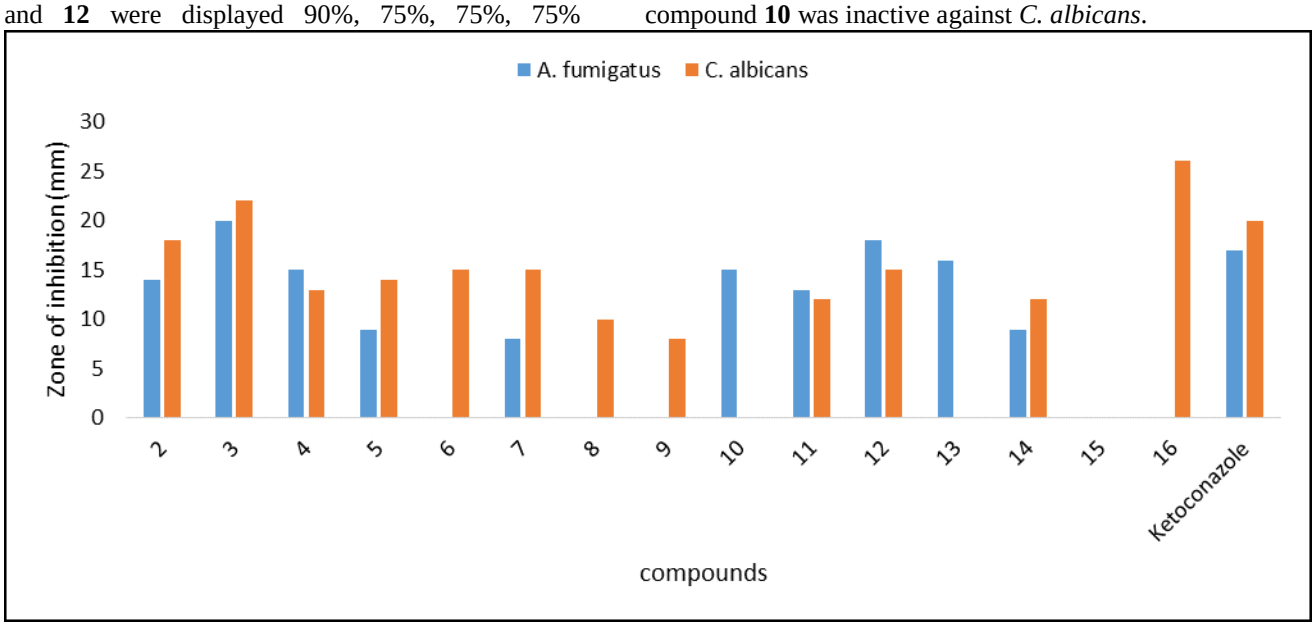


Figure 5. Preliminary antifungal activity versus pyrazole derivatives **2-16.**

Figure 6 displayed broad antifungal spectrum against the screened fungi for the prepared compounds **17-29**. Interestingly, the compound **18** was displayed antifungal activity more than ketoconazole against *C. albicans* and *A. fumigatus* which reach to 125% and 141%, respectively. Moreover, compounds **19** and **20** were displayed 100% and 94% antifungal activity by comparison with ketoconazole against *A. fumigatus*. While, compounds **23**, **24**, **26** and **17** were displayed 76%, 71%, 59% and 59%, antifungal activity by comparison with ketoconazole against *A. fumigatus*. Unfortunately, compounds **21**, **22**, **25**, **27**, **28** and **29** were inactive against A. fumigatus. On the other hand, the compound **21** was displayed antifungal activity more than ketoconazole against *C. albicans* which reach to 135%. Moreover, compounds **19**, **20**, **22**, **25** and **29** were displayed 100%, 90%, 90%, 90%, 95% antifungal activity by comparison with ketoconazole against *C. albicans*. While, compounds **23**, **24**, **27** and **28** were displayed 70%, 65%, 70% and 75% antifungal activity by comparison with ketoconazole against *A. fumigatus*. Unfortunately, compounds **17** and **26** were inactive against *C. albicans.*

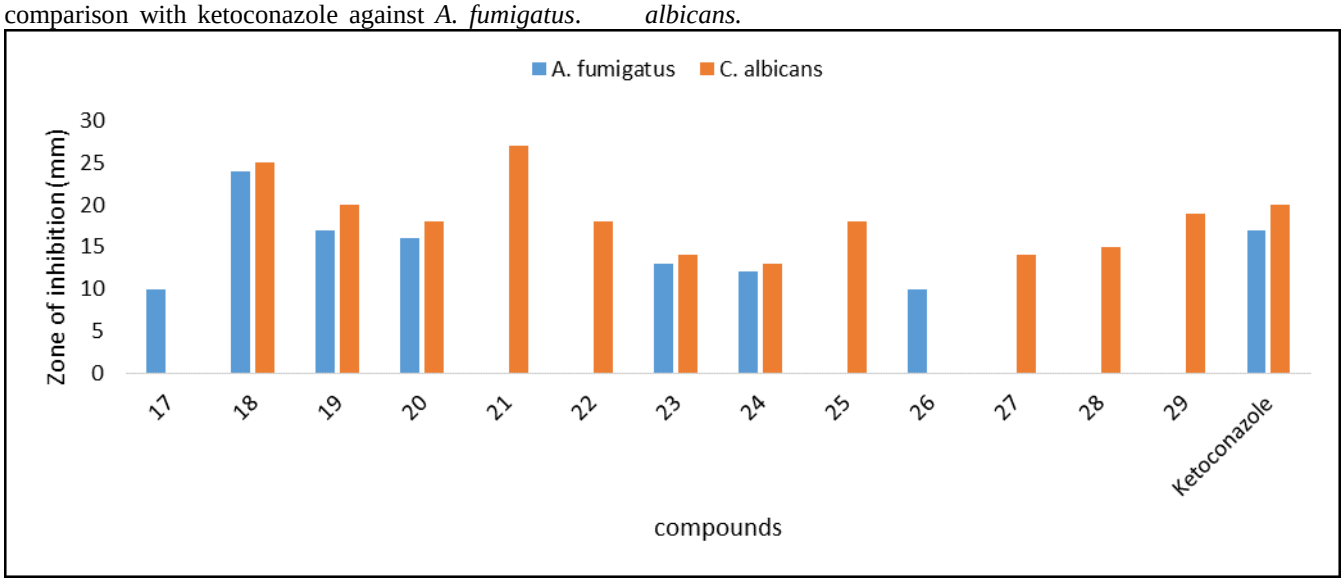


Figure 6. Preliminary antifungal activity versus pyrazole derivatives **17-29**

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Transmission Electron Microscopy

In TEM observations, there were clear cellular alterations of stained ultrathin sections (70 nm) of all tested organisms. According to ultra-thin sections of treated *A. fumigatus* represented completely deformations of both cell wall and cell membrane with disappearance of the cytoplasmic materials Fig. 7B, compared with control ultra-structures Fig. 7A; intact cell wall (CW) and cell membrane (CM) with identified organelles; mitochondria (M) and nucleus (N). In case of ultra-thin sections of treated C. albicans with compound **21** represented interference of the compound upon the structure of yeast with alterations of the cell wall which became irregular with changed in cytoplasmic membrane; increasing in thickness and the cytoplasmic materials constricting in the center of the cell Fig. 7D, compared with control cell; showed identified rounded cell with intact cell wall (CW), cell membrane (CM), Mitochondria (M) and; vacuole (V) Fig. 7C. While, compounds **2** and **9** have shown great antibacterial activities against *S. aureus*; alterations in cell structure resulted in membrane damage which appeared to have ruptured potentially with leaks of intracellular materials and cellular damage leads finally to complete cell deformation Fig. 7F and 7G, respectively, compared with control cell; Fig. 7E. The affected cells of *B. subtilis* with compounds **3**, **12** and **19** represented that all cells were lysed and void of cytoplasmic fluid with completely shrinkage of cytoplasmic membrane Fig. 7I – 7K, compared with control cell Fig. 7H, which showed a rod-shaped structure with an undamaged and intact outer membrane. Untreated cells of *E. coli* showed a uniformly dense and homogeneous microstructure Fig. 7L, while, the cells affected with compound **19** revealed less electron dense materials with disappearance of cytoplasmic materials Fig. 7M. Untreated cells of P. vulgaris had normal cell condition with spherical shape and rigid surface with cytoplasm; continuously in close contact with the cell wall with normal intact with cytoplasmic membrane, Fig. 7N, since, completely cellular damages; breaking in cell wall and leaking in cytoplasmic materials were noticed after treatment with compound **12** which illustrated in Fig. 7O. According to A. fumigatus, compound **18** was the most active compound that recorded 24 mm Fig. 7A. However, compound **21** represented the highly remarked inhibition zone against *C. albicans*; 27 mm Fig. 7B, compared with antifungal reference used; ketoconazole; 17 mm and 20 mm, respectively. In case of compounds **2** and **19** had the same value against *S. aureus*; 16mm Fig. 7A and 7B, also, compounds **3**, **12** and **19** had the same inhibition value against *B. subtilis*; 19mm Fig. 7C and 7D, since, *E. coli* was mostly affected with compound **19**; 17 mm Fig. 7A, while, *P. vulgaris* was affected with compound 12; 24mm Fig. 7B; compared with antibacterial reference used; gentamycin; 24, 26, 30 and 25mm, respectively.

Structure activity relationship (SAR):

The potent derivatives antibacterial activities **2**, **3**, **12**, and **19** were related to the ability of the compounds to affect the cell wall of the bacterial via interacting with the peptidoglycan layer. These interactions afforded protons fluxing which make changes in the cell membrane and cell wall and until cell death. This illustration was confirmed by using a TEM micrograph, which revealed to the cell membrane and cell wall rupture in the tested bacteria treated with the used derivatives. The activity of compounds **2**, **3** may be related to the cyano group presence at position 4 of the pyrazole ring which is an electron-withdrawing group, in addition to the two carbonyl oxygen presence at positions 2 and 5 of compound **2** and also the fused benzene ring in compound **3**. However, the activity of compound **12** may be related to the chlorine atom presence at position 4 of the pyrimidine ring. Replacement of hydrogen with the methyl group at position 2 of pyrimidine ring exhibited potent activity. As regard compound **19**, it showed good activity against both bacterial strains, this may be due to the free -SH group presence in addition to the carbonyl and (-NHNH-) groups in the side chain which produce a flux of protons that interrupt the cell wall and cell membrane chemical structure leading to cell rupture. Interestingly, the addition of -CS-SH moiety to compound **19** may be the main reason for its activity. The activity of compounds **18** and **21** may be related to their hydrazine ($NHNH_2$) moiety in addition to the presence of the fused heterocyclic rings.

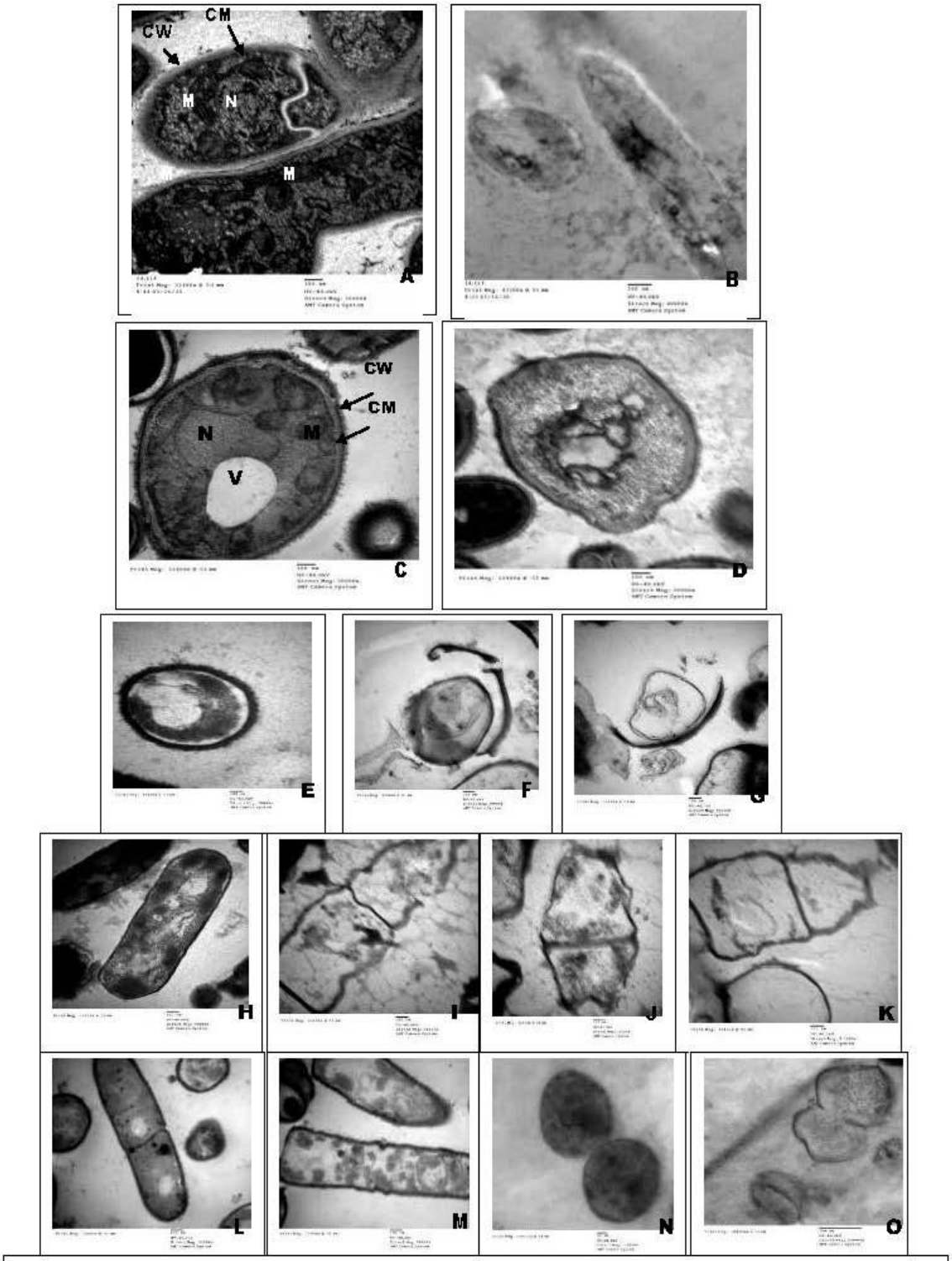

Figure [7]: TEM micrographs of *A. fumigatus*; A: control and B: affected with compound 18; C: control of ***Candida albicans*** and D: affected with compound 21; E: control of ***S. aureus***, F and G: affected with compounds 2 and 19; H: control of ***B. subtilis*** and I, J, K : affected with compounds 3, 12 and 19; L: control of ***E. coli*** and M: affected with compound 19; N: control of ***P. vulgaris*** and O: affected with compound 12.

Conclusion:

5-amino-1,3-diphenyl-1H-pyrazole-4-carbonitrile **1** was used for preparation of some pyrazole derivatives like as: 2,5-dioxopyrrole, 1,3-dioxoisoindoline, N-acetamide, N-benzenesulfonamide, pyrazolopyridine, pyrazolopyridine-2-one, pyrazolopyrimidin-4-one, pyrazolopyrimidine-4-thione, pyrazolopyrimidine, pyrazolotriazolopyrimidine, 1,3,4-oxadiazol, pyrazolopyrazole. Evaluated the prepared compounds antimicrobial activities against *C. albicans, A. fumigatus, B. subtilis, S. aureus, P. vulgaris and E.* coli with using Transmission Electron microscopic investigation for compounds **2**, **18**, **19**, **20**, **23** and **24** showed highly effect against all the tested microorganisms.

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