Synthesis and biological activity of a new class of enaminonitrile pyrazole

Sabah S. Mohamed 1, Nadia T. Dauoud 2, Sara N. Shaban 2, Nahed F. Abdelghaffar 2,
Galal H. Sayed 3, Kurls E. Anwer 3,*

1 The Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.
2 Faculty of Science, Al-Azhar University (for girls), Nasr City, Cairo, Egypt.
3 Heterocyclic Synthesis Lab., Chemistry Department, Faculty of Science, Ain Shams University, Abbassia, 11566, Cairo, Egypt.

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 sulfonfyl chloride to afford the pyrazole derivatives 2-5. The treatment of pyrazole derivative 1 with some active methylene reagents namely: malononitrile, cyanoacetamide, ethyl acetocetate afforded the pyrazolopyridine 6-9. Reaction of compound 1 with acetic anhydride gave the pyrazolopyrimidinone 10, which was allowed to react with P2S5 and POCl3 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, 13C-NMR, 1H-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], antihistaminic [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”, 1H-NMR and 13C-NMR spectra have been recorded at 300 MHz on a “Varian Mercury spectrometer” using DMSO as an internal standard in using DMSO-d6. 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 filtered, and then recrystallized from methanol gave compound 2.

Yield 75%; pale yellow crystal; m.p. 180-182 °C; IR (KBr, ν, cm⁻1) 2228 (CN), 1725, 1659

*Corresponding author e-mail: kurlsekram@sci.asu.edu.eg; (Kurls E. Anwer).
Receive Date: 13 February 2021, Revise Date: 22 February 2021, Accept Date: 01 March 2021
DOI: 10.21608/ejchem.2021.62916.3350
©2021 National Information and Documentation Center (NIDOC)
(CO), 1593 (C=O). 1H-NMR (DMSO-d6) δ: 6.74-7.86 (m, 12H; Ar-H); MS: m/z 340 (M+1) (30.6%). Anal. Calcd. for C16H12N4O2: 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-dioxoisodolin-2-yl)-1,3-diphenyl-1H-pyrazole-4-carbonitrile (3)

To a hot solution of enaminoester 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 4h. 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 °C; IR (KBr, cm⁻¹): 2227 (CN), 1784, 1725 (CO), 1592 (C=N); 1H-NMR (DMSO-d6): δ: 6.75-8.54 (m, 14H; Ar-H); MS: m/z 390 (M+1) (38.5%). Anal. Calcd. for C16H12N4O2: 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 enaminoester 1 (2.6 g, 0.01 mol.) in dioxane (25 mL), acetyl chloride (1.22 mL, 0.01 mol.) and drops of TEA was 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 °C; IR (KBr, cm⁻¹): 3288 (NH), 2233 (CN), 1633, 1601 (C=O), 1593 (C=O); 1H-NMR (DMSO-d6): δ: 2.27 (s, 3H; CH3), 6.75-7.87 (m, 10H; Ar-H), 10.81 (s, 1H, NH; D2O exchangeable), 13C-NMR (DMSO-d6): δ: 6.82, 117.2, 123.9, 130.7, 133.0, 134.2, 140.4, 141.6, 144.5, 150.2 and 159.3; MS: m/z 302 (M+1) (4.6%). Anal. Calcd. for C16H14N4O2: 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 enaminoester 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 °C; IR (KBr, cm⁻¹): 3201 (NH), 2204 (CN), 1592 (C=N); 1H-NMR (DMSO-d6): δ: 6.73-7.88 (m, 15H; Ar-H), 12.17 (s, 1H, NH; D2O exchangeable); MS: m/z 400 (M+1) (26.0%). Anal. Calcd. for C22H13N4O2S: 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 enaminoester 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. 101-102 °C; IR (KBr, cm⁻¹): 3442, 3315, 3290 (NH), 2169 (CN), 1633, 1601 (C=O). 1H-NMR (DMSO-d6): δ: 6.72-7.87 (m, 10H; Ar-H), 10.32 (s, 4H, 2NH2; D2O exchangeable); MS: m/z 326 (M+1) (40.9%). Anal. Calcd. for C16H14N6O: 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 enaminoester 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 °C; IR (KBr, cm⁻¹): 3445, 3309, 3281 (NH2), 1658 (C=O), 1595 (C=N); 1H-NMR (DMSO-d6): δ: 6.72-7.86 (m, 10H; Ar-H), 8.89 (s, 4H, 2NH2; D2O exchangeable), 11.24 (s, 2H, CONH2; D2O exchangeable); 13C-NMR (DMSO-d6): δ: 117.1, 123.9, 130.7, 133.0, 134.2, 140.9, 141.6, 142.4, 144.7, 150.2 and 159.3; MS: m/z 344 (M+1) (13.3%). Anal. Calcd. for C16H14N6O: C, 66.27; H, 4.68; N, 24.40; Found: C, 66.65; 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 enaminoester 1 (2.6 g, 0.01 mol.) in ethanol (25 mL), ethyl acetocetate (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 °C; IR (KBr, cm⁻¹): 3423, 3311 (NH2), 1635 (C=O), 1591 (C=N); 1H-NMR (DMSO-d6): δ: 1.22 (t, J = 6.2 Hz, 3H; CH3CH2), 4.02 (s, 3H; CH3); 4.20 (q, J = 6.4 Hz, 2H; CH2CH3), 6.72-7.87 (m, 10H; Ar-H), 9.69 (s, 2H, NH; D2O exchangeable); 13C-NMR (DMSO-d6): δ: 18.4, 23.2, 57.6, 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+1) (24.2%). Anal. Calcd. for C22H20N4O2: C, 70.95;
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 3 h. 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 °C; IR (KBr, ν cm⁻¹) 3149, 3312 (NH₂), 2258 (CN), 1746 (C=O), 1631 cm⁻¹ (C=N); ¹H-NMR (DMSO-d₆) δ: 6.72-7.87 (m, 10H; Ar-H), 8.52 (s, 2H, NH₂; D₂O exchangeable), 11.47 (s, 1H, NH; D₂O exchangeable); ¹³C-NMR (DMSO-d₆) 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 C₉H₁₁N₂O: 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]pyrimidine-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 4 h. 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 °C; IR (KBr, ν cm⁻¹) 3236 (NH), 1681 (C=O), 1608 (C=N); ¹H-NMR (DMSO-d₆) δ: 2.52 (s, 3H; CH₃), 6.72-7.87 (m, 10H; Ar-H), 11.57 (s, 1H, NH; D₂O exchangeable); ¹³C-NMR (DMSO-d₆) 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 C₁₀H₁₁N₂O: 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 6 h. 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 °C gave compound 11.

Yield 76%; black crystal; m.p. 150-152 °C; IR (KBr, ν cm⁻¹) 3245 (NH), 1593 (C=N), 1263 (C=S); ¹H-NMR (DMSO-d₆) δ: 2.50 (s, 3H; CH₃), 6.74-7.86 (m, 10H; Ar-H), 8.62 (s, 1H, NH; D₂O exchangeable); ¹³C-NMR (DMSO-d₆) δ: 24.3, 110.6, 117.2, 123.7, 130.3, 133.4, 133.6, 143.2, 141.0, 141.3, 150.6 and 172.6; MS: m/z 318 (M⁺) (64.2%). Anal. Calcd. for C₁₀H₈N₂S: 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 3 h. The reaction mixture after cooling was poured into ice water, the solid formed was filtrated, and then recrystallized from petroleum ether 60-80 °C gave compound 12.

Yield 80%; black crystal; m.p. 120-122 °C; IR (KBr, ν cm⁻¹) 1622, 1595 (C≡N); ¹H-NMR (DMSO-d₆) δ: 2.49 (s, 3H; CH₃), 6.74-7.84 (m, 10H; Ar-H); ¹³C-NMR (DMSO-d₆) δ: 24.3, 117.2, 123.7, 130.3, 133.0, 133.4, 133.6, 141.0, 141.3, 150.7 and 154.5; MS: m/z 320 (M⁺) (41.45%). Anal. Calcd. for C₁₀H₁₁N₂Cl: 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 8 h. 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 8 h. 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 °C; IR (KBr, ν cm⁻¹) 3447, 3357 (NH₂), 3218, 3189 (NH), 1633, 1612 (C≡N), 1266 (C=S); ¹H-NMR (DMSO-d₆) δ: 2.51 (s, 3H; CH₃), 6.74-7.86 (m, 10H; Ar-H), 8.24 (s, 1H, NH; D₂O exchangeable), 8.87 (s, 1H, NH; D₂O exchangeable), 9.74 (s, 2H, NH); D₂O exchangeable); ¹³C-NMR (DMSO-d₆) 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 C₁₀H₁₁N₂S: 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 20 h. 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 20 h. 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 20 h. The reaction mixture after cooling, the solid formed was filtrated, and then recrystallized from acetone produced compound 14.
formation of compound 12 (3.2 g, 0.01 mol.) in pyridine (15 mL), p-aminoacetoephene (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 °C; IR (KBr, ν cm⁻¹) 3200 (NH), 1655 (C=O), 1595 (C=N). ¹H-NMR (DMSO-d₆) δ: 2.50 (s, 3H; CH₃), 3.13 (s, 3H; COCH₃), 6.24 (s, 1H, NH; D₂O exchangeable), 6.74-7.86 (m, 14H, Ar-H); ¹³C-NMR (DMSO-d₆) 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 C₂₀H₁₈N₄O: 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]pyrimidin (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 °C; IR (KBr, ν cm⁻¹) 3419 (NH), 3212 (NH), 1594 (C=N). ¹H-NMR (DMSO-d₆) δ: 2.50 (s, 3H; CH₃), 6.30 (s, 2H, NH₂; D₂O exchangeable), 6.7-7.87 (m, 10H; Ar-H), 7.94 (s, 1H, NH; D₂O exchangeable); MS: m/z 316 (M⁺) (20.1%). Anal. Calcd. for C₁₉H₁₆N₄O: 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 °C; IR (KBr, ν cm⁻¹) 3420 (O=C=O), 1650 (C=O), 1572 (C=N). ¹H-NMR (DMSO-d₆) δ: 1.8 (t, J = 6.6 Hz, 3H; CH₃), 2.50 (s, 3H; CH₃), 4.27 (q, J = 7.2 Hz, 2H; CH₂CH₂), 4.52 (s, 2H; OCH₂CO), 6.75-7.84 (m, 10H; Ar-H); ¹³C-NMR (DMSO-d₆) δ: 15.6, 22.1, 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 C₂₀H₂₃NO₂: 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 °C; IR (KBr, ν cm⁻¹) 3424 (NH₂), 3273 (NH), 1661 (C=O), 1600 (C=N); ¹H-NMR (DMSO-d₆) δ: 2.51 (s, 3H; CH₃), 4.27 (s, 2H; OCH₂CO), 6.39 (s, 1H, NH; D₂O exchangeable); 6.76-7.86 (m, 10H; Ar-H), 9.28 (s, 2H, NH₂; D₂O exchangeable); MS: m/z 374 (M⁺) (25.6%). Anal. Calcd. for C₂₀H₁₈N₄O₂: 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-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)acetyl)hydrazine-1-carboxylic 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 °C; IR (KBr, ν cm⁻¹) 3440 (NH), 2564 (SH), 1658 (C=O), 1601, 1592 (C=N), 1259 (C=S); ¹H-NMR (DMSO-d₆) δ: 1.95 (s, 1H, SH; D₂O exchangeable), 2.50 (s, 3H; CH₃), 4.13 (s, 2H; OCH₂CO), 6.68-7.87 (m, 10H; Ar-H), 9.62 (s, 1H, NH; D₂O exchangeable), 10.32 (s, 1H, NH; D₂O exchangeable); MS: m/z 450 (M⁺) (5.3%). Anal. Calcd. for C₁₉H₁₈N₄O₂S: 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%.

*Egypt. J. Chem.*** 64, No. 6 (2021)*
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 filtered, and then recrystallized from ethanol gave compound 20.

Yield 81%; red crystal; m.p. 120-122 °C; IR (KBr, ν, cm⁻¹) 2529 (SH), 1599, 1591 (C=O). ¹H-NMR (DMSO-d₆) δ: 2.51 (s, 3H; CH₃), 3.78 (s, 1H, SH; D₂O exchangeable), 4.14 (s, 2H; OCH₂CO), 6.72-7.84 (m, 10H; Ar-H); ¹³C-NMR (DMSO-d₆) 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, 130.1, 136.4 (M⁺) (31.6%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: 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 a new class of enamino nitrile pyrazole (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 filtered, and then recrystallized from benzene gave compound 21.

Yield 76%; beige crystal; m.p. 280-282 °C; IR (KBr, ν, cm⁻¹) 3347, 3300 (NH), 3225 (NH), 1599, 1591 (C=N); ¹H-NMR (DMSO-d₆) δ: 2.50 (s, 3H; CH₃), 4.19 (s, 2H; OCH₂CO), 6.72-7.86 (m, 10H; Ar-H), 8.72 (s, 1H, NH; D₂O exchangeable), 10.29 (s, 2H, NH; D₂O exchangeable); MS: m/z 414 (M⁺) (15.0%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: C, 60.86; H, 4.38; N, 27.04; Found: C, 60.91; H, 4.41; N, 27.04; 26.89%.

2.1.21. Formation of 5-((6-methyl-1,3-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)-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 waater, the solid formed was filtrated, and then recrystallized from ethanol gave compound 22.

Yield 81%; golden yellow crystal; m.p. 140-142 °C; IR (KBr, ν, cm⁻¹) 3385, 3313 (NH), 3191 (C=O), 1627, 1592 (C=N); ¹H-NMR (DMSO-d₆) δ: 6.73-7.87 (m, 10H; Ar-H), 8.68 (s, 1H, NH; D₂O exchangeable), 10.31 (s, 2H, NH₂; D₂O exchangeable); MS: m/z 275 (M⁺) (8.1%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: C, 69.80; H, 4.76; N, 25.44; Found: C, 69.67; H, 4.88; N, 25.45%.

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

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

Yield 75%; brown crystal; m.p. 146-148 °C; IR (KBr, ν, cm⁻¹) 3441 (OH), 3211 (NH), 2203 (CN), 1731 (C=O), 1591 (C=N); ¹H-NMR (DMSO-d₆) δ: 4.27 (s, 2H; NHCH₂CO), 6.72-7.87 (m, 10H; Ar-H), 9.53 (s, 1H, NH; D₂O exchangeable), 12.57 (s, 1H, OH; D₂O exchangeable); MS: m/z 318 (M⁺) (22.2%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: 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 enamino nitrile 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 °C; IR (KBr, ν, cm⁻¹) 3476 (OH), 3414, 3311 (NH), 3223 (NH), 1709 (C=O), 1645, 1593 (C=N); ¹H-NMR (DMSO-d₆) δ: 5.08 (s, 2H, NH; D₂O exchangeable), 6.72-7.89 (m, 10H; Ar-H), 10.0 (s, 1H, NH; D₂O exchangeable), 10.40 (s, 1H, OH; D₂O exchangeable); MS: m/z 318 (M⁺) (9.8%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: 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-dihydropyrrolo[3,4-c]pyrazol-3-amine (25)

To a hot solution of enamino nitrile 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 °C; IR (KBr, ν, cm⁻¹) 3385, 3313 (NH), 3201 (NH), 1627, 1592 (C=N); ¹H-NMR (DMSO-d₆) δ: 6.73-7.87 (m, 10H; Ar-H), 8.68 (s, 1H, NH; D₂O exchangeable), 10.31 (s, 2H, NH₂; D₂O exchangeable); MS: m/z 275 (M⁺) (8.1%). Anal. Calcd. for C₁₄₂H₁₄₂N₂O₂S: 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 enamino nitrile 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 filtered, and then recrystallized from toluene afforded compound 26.

Yield 76%; red crystal; m.p. 106-108 O C; IR (KBr, v cm-1) 3456, 3312 (NH2 NH), 1688 (C=C), 1611 (C=N); 1H-NMR (DMSO-d6) δ: 6.69-7.92 (m, 10H; Ar-H), 9.55 (s, 2H; NH2; D2O exchangeable), 10.28 (s, 1H; NH; D2O exchangeable); 13C-NMR (DMSO-d6) 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 C12H11N3O: C, 76.43; H, 5.28; 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 enammonitrite 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 filtered, and then recrystallized from toluene produced compound 27

Yield 75%; red crystal; m.p. 220-222 O C; IR (KBr, v cm-1) 3397, 3311 (NH2), 3200 (NH), 1600, 1593 (C=N), 1287 (C=S); 1H-NMR (DMSO-d6) δ: 6.72-7.87 (m, 10H, Ar-H), 10.33 (s, 2H, NH2; D2O exchangeable), 13.14 (s, 1H, NH, D2O exchangeable). 13C-NMR (DMSO-d6) δ: 117.2, 123.9, 130.7, 133.0, 133.8, 134.2, 140.9, 141.6, 150.5 and 189.1; MS: m/z 319 (M+) (10.7%). Anal. Calcd. for C12H11N3S: C, 73.77; H, 4.12; 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 enamnonitrite 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 filtered, and then recrystallized from ethanol produced compound 28.

Yield 81%; brown crystal; m.p. 138-140 O C; IR (KBr, v cm-1) 1633, 1600 (C=N). 1H-NMR (DMSO-d6) δ: 1.31 (t, J = 6.0 Hz, 3H; CH2CH3), 4.20 (q, J = 6.6 Hz, 2H; CH2CH3), 6.72-7.86 (m, 15H; Ar-H); 13C-NMR (DMSO-d6) 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 C15H15N3O: 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]pyrimidine-4-amine (29)

To a hot solution of enamnonitrite 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 filtered, and then recrystallized from methanol produced compound 29.

Yield 85%; red crystal; m.p. 160-162 O C; IR (KBr, v cm-1) 3427, 3311 (NH2), 1591 (C=N); 1H-NMR (DMSO-d6) δ: 6.72-7.86 (m, 2OH; Ar-H), 9.41 (s, 2H, NH2; D2O exchangeable), 10.57 (s, 2H, NH2; D2O exchangeable). 13C-NMR (DMSO-d6) 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 C15H14N5O: 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 agar plates fungi using malt agar “fungi using the malt broth”. Sterile swabs were immersed in the crossing and used to vaccine. Mueller-Hinton 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°C after 48h “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, 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 υNH2 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 ¹H-NMR spectrum revealed bands at 2.27 and 10.81 ppm corresponding to CH₃ and NH protons, respectively. Its ¹³C-NMR spectrum revealed signals at 20.4 and 159.3 ppm corresponding to CH₃ and C=O, respectively. Its molecular ion peak appeared at m/z 302 (M⁺) in the mass spectrum which corresponding to C₁₈H₁₄N₄O.

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

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 CONH₂ 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 υC=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$_2$S 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-aminocacetophenone 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 pyrazolopyrimidine-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 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). Its $^1$H-NMR spectrum exposed bands corresponding to OCH$_2$CO, SH, and NH proton at 4.19, 12.84, and 13.33 ppm, respectively. Its molecular ion peak appeared at m/z=414 [M$^+$] in mass spectrum which corresponding to C$_{21}$H$_{18}$N$_8$O$_2$.

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$_7$OS. (Scheme 4).

Finally, (Scheme 5) the showed synthesis of bioactive heterocycle derivatives via reaction of enammonitrile 1 with different electrophilic and nucleophilic reagents. Refluxing enammonitrile 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, enammonitrile 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 pyrazolopyrimidine-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

**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 “Bacillus 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:

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>A. fumigatus</th>
<th>C. albicans</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>P. vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>NT</td>
<td>17</td>
<td>20</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>NT</td>
<td>NT</td>
<td>24</td>
<td>26</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>16</td>
<td>11</td>
<td>NA</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>10</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>22</td>
<td>NA</td>
<td>19</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>13</td>
<td>NA</td>
<td>14</td>
<td>NA</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>14</td>
<td>NA</td>
<td>10</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>15</td>
<td>NA</td>
<td>9</td>
<td>8</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>15</td>
<td>NA</td>
<td>13</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
<td>10</td>
<td>NA</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NA</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>12</td>
<td>NA</td>
<td>10</td>
<td>NA</td>
<td>16</td>
</tr>
<tr>
<td>11</td>
<td>18</td>
<td>15</td>
<td>NA</td>
<td>19</td>
<td>NA</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>8</td>
<td>NA</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>NA</td>
<td>26</td>
<td>NA</td>
<td>8</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>17</td>
<td>24</td>
<td>25</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>18</td>
<td>17</td>
<td>20</td>
<td>16</td>
<td>19</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>19</td>
<td>16</td>
<td>18</td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>NA</td>
<td>27</td>
<td>NA</td>
<td>14</td>
<td>NA</td>
<td>14</td>
</tr>
<tr>
<td>21</td>
<td>NA</td>
<td>18</td>
<td>NA</td>
<td>10</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>22</td>
<td>13</td>
<td>14</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>23</td>
<td>12</td>
<td>13</td>
<td>10</td>
<td>14</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>NA</td>
<td>18</td>
<td>NA</td>
<td>17</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>NA</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>26</td>
<td>NA</td>
<td>14</td>
<td>NA</td>
<td>8</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>27</td>
<td>NA</td>
<td>15</td>
<td>NA</td>
<td>11</td>
<td>NA</td>
<td>13</td>
</tr>
<tr>
<td>28</td>
<td>NA</td>
<td>19</td>
<td>NA</td>
<td>14</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>29</td>
<td>NA</td>
<td>19</td>
<td>NA</td>
<td>14</td>
<td>NA</td>
<td>12</td>
</tr>
</tbody>
</table>

* 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.
SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW CLASS OF ENAMINONITRILE PYRAZOLE

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.

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

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

Egypt. J. Chem. 64, No. 6 (2021)
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.

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.
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
Transcription 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 ultrathin 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 (NHNH₂) 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 C. albicans and D: affected with compound 21; E: control of S. aureus; F and G: affected with compounds 9 and 19; H: control of B. subtilis and I and 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-dioxoisindoline, 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.

Acknowledgement

The authors thank Dr. Mona Abd El-Gelel Mohamed, professor of Biochemistry Division, chemistry department, Faculty of Science, Al-Azhar University, Egypt, and Dr. Basma Hamdy Amin, Associate professor of Microbiology at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt, for kindly providing explanation assistance during writing the antimicrobial evaluation, SAR and TEM ultrastructure observations of tested organisms. Also, the authors thank Mr. Ahmed Abbas, researcher at the Drugs Department, Faculty of Pharmacy, Mansoura University, Egypt, for kindly providing technical assistance during the antitumor activity testing.

Reference:


