

Article

Synthesis and Antimicrobial Evaluation of Some Novel Bis- α,β -Unsaturated Ketones, Nicotinitrile, 1,2-Dihydropyridine-3-carbonitrile, Fused Thieno[2,3-*b*]pyridine and Pyrazolo[3,4-*b*]pyridine Derivatives

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Abstract: The title compounds were prepared by reaction of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**) with different aromatic aldehydes **2a–c**, namely Furfural (**2a**), 4-chlorobenzaldehyde (**2b**) and 4-methoxybenzaldehyde (**2c**) to yield the corresponding α,β -unsaturated ketones **3a–c**. Compound **3** was reacted with malononitrile, 2-cyanoacetamide or 2-cyanothioacetamide yielded the corresponding bis[2-amino-6-(aryl)nicotinitrile] **4a–c**, bis[6-(2-aryl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] **5a–c** or bis[6-(2-aryl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile] **6a,b**, respectively. The reaction of compound **6a** with each of 2-chloro-*N*-(4-bromophenyl)acetamide (**7a**), chloroacetamide (**7b**) in ethanolic sodium ethoxide solution at room temperature to give the corresponding 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis-6-(2-furyl)thieno[2,3-*b*]pyridine-2-carboxamide] derivatives **9a,b**. While compound **6a** reacted with hydrazine hydrate yielded the 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine] **11**. The structures of the products were elucidated based on their spectral properties, elemental analyses and, wherever possible, by alternate synthesis. Antimicrobial evaluation of the products was carried out.

Keywords: heterocycles; α,β -unsaturated ketones; pyrazoles; antimicrobial activity

1. Introduction

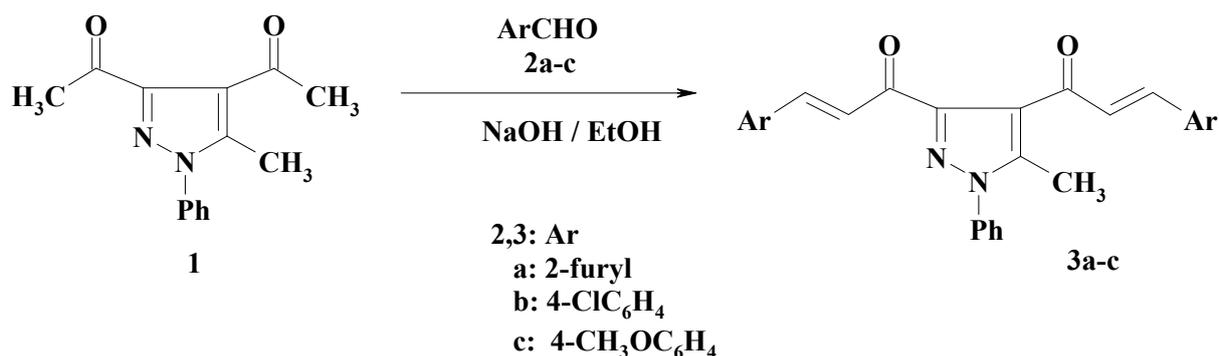
Recently, the synthesis of a wide variety of bis heterocyclic compounds of different ring sizes with one or several heteroatoms has received a great deal of attention, not only as model compounds for main chain polymers but also because many biologically active natural and synthetic products have molecular symmetry [1–8]. In addition, α,β -unsaturated ketones (Chalcones) derivatives have a variety of pharmacological activities such as antimalarial [9], anticancer [10–13], anti-inflammatory [14], antibacterial [15], antifungal agents [16]. Some nicotinonitrile derivatives are used as non-linear optical (NLO) materials [17], electrical materials [18]. Others have found uses as anticancer agents and Antimicrobial activity [19]. In addition, dihydropyridine derivatives display a broad spectrum of medicinal activities, mainly as antihypertensive and antiarrhythmic drugs [20–24]. Others have found uses as anticancer agents [25–27].

On the other hand, many compounds with pyrazole ring are of interest due to their broad spectrum of biological activities against NOS inhibitor [28], monoamine oxidase inhibitor [29], and antibacterial [30], antiameobic [31]. Moreover, *N*-phenylpyrazole derivatives play an important role in antitumor screening [32] as well as potent antimicrobial activity [33,34]. Furthermore, a pyrazolo[3,4-*b*]pyridines have Potential and selective inhibitors of glycogen synthase kinase-3 (GSK-3) [35]. Also 3-Cyano-2(1*H*)-pyridinethiones [36,37] and their related compounds were found to be very reactive substances for the synthesis of many different heterocyclic systems which exhibited biological activities such as antibacterial and antifungal [38]. In light of these findings, we report here the synthesis of some novel bis-heterocycles containing *N*-phenylpyrazole as a base unit. In addition, some of the newly synthesized compounds were screened for their antibacterial and antifungal activities.

2. Results and Discussion

α,β -Unsaturated ketons (chalcones) are active intermediates and excellent starting materials for the synthesis of several heterocyclic systems. Thus, a Claisen-Schmidt reaction of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**), prepared following the previously reported methods [39–41], with Furfural (**2a**), 4-chlorobenzaldehyde (**2b**) and 4-methoxybenzaldehyde (**2c**), in 10% ethanolic sodium hydroxide afforded the corresponding bis(α,β -unsaturated ketons) **3a–c** in 65, 95 and 50% yields, respectively (Scheme 1) [42].

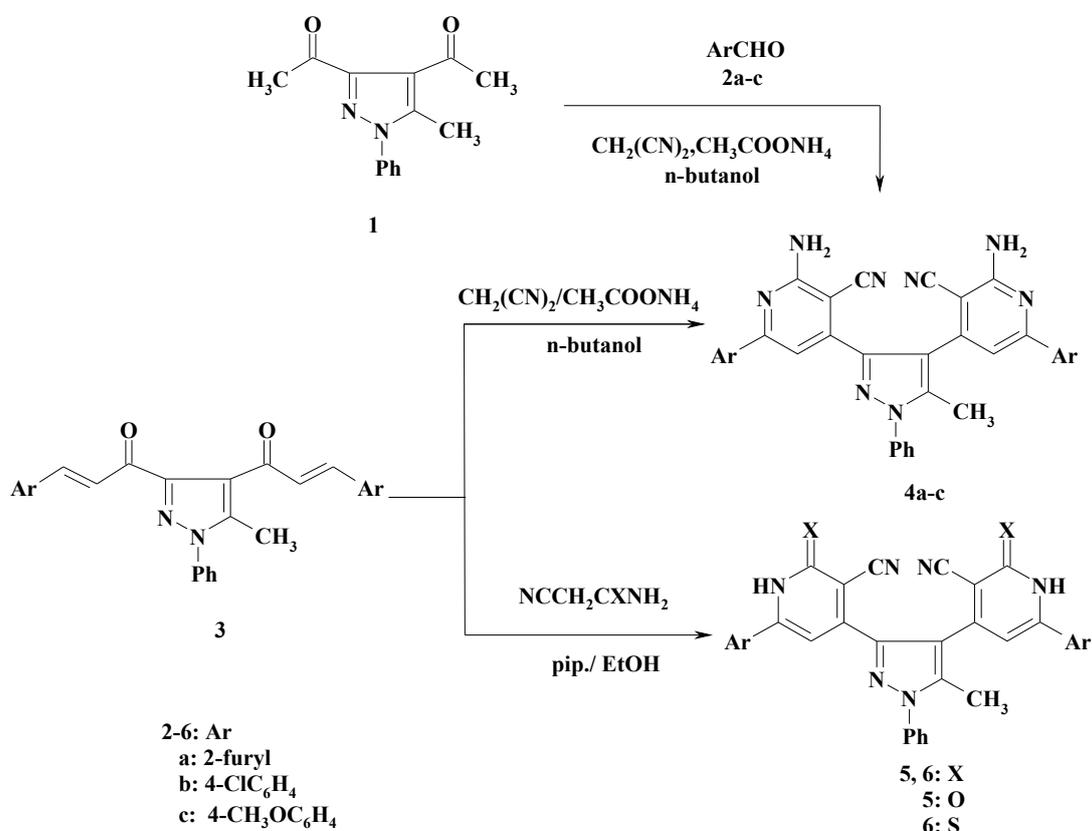
Scheme 1. Synthesis of α,β -unsaturated ketones **3a–c**.



The structure of the products **3a–c** was determined from spectroscopic as well as elemental analytical data. The IR spectra of **3a–c** showed the appearance of carbonyl function absorption bands in the region 1663–1658 cm^{-1} . The $^1\text{H-NMR}$ spectra of **3a–c** displayed the two signals of olefinic protons besides the aromatic protons [42]. Thus, compound **3b**, taken as a typical example of the prepared series, revealed absorption bands at 2923, 1663 and 1598 cm^{-1} corresponding to CH-aliphatic, two carbonyl groups and C=C functions, respectively. Its $^1\text{H-NMR}$ spectrum showed signals at δ 2.38 due to CH_3 protons, four duplet signals at δ 7.24 ($J = 11.3$ Hz), 7.38 ($J = 10.5$ Hz), 7.54 ($J = 10.5$ Hz), 7.62 ($J = 11.3$ Hz) due to 4 CH = protons, in addition to an aromatic multiplet protons in the region δ 7.66–7.84. Their mass spectra revealed in each case the respective molecular ion peak.

Next, condensation of compounds **3a–c** with malononitrile in the presence of ammonium acetate in *n*-butanol afforded the corresponding 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl) bis[2-amino-6-(aryl)nicotinonitrile] **4a–c** were also prepared in fair yields by applying the aforementioned one pot reaction of the compound **1**, the corresponding aldehyde and malononitrile in the presence of ammonium acetate (Scheme 2).

Scheme 2. Synthesis of bis[2-amino-6-(aryl)nicotinonitrile] **4a–c**, bis[6-(2-aryl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] **5a–c** and bis[6-(2-aryl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile] **6a,b**.

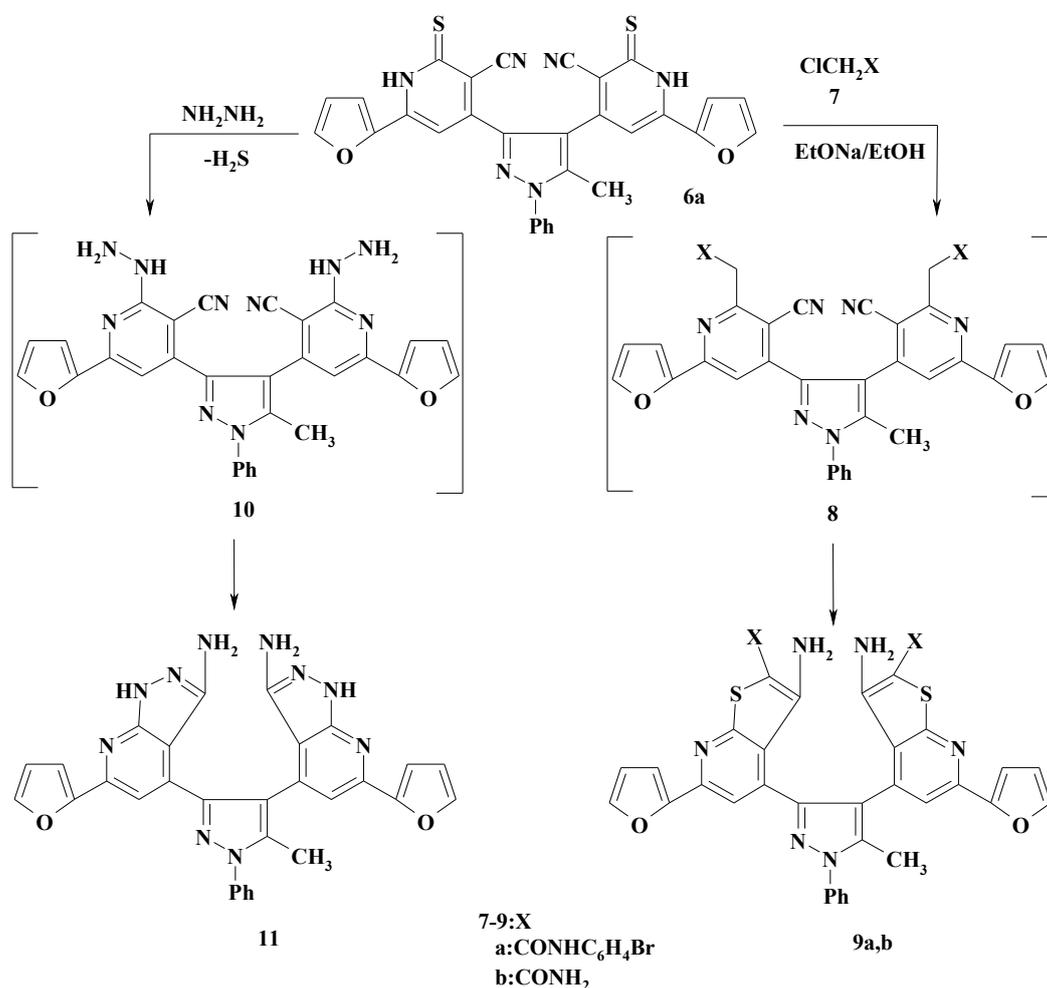


The structures of the newly synthesized compounds were confirmed on the basis of their elemental analysis and IR, $^1\text{HNMR}$ and mass spectral data. The IR spectrum of compound **4b**, taken as a typical example of the series prepared, revealed absorption bands at 2210 and 3438, 3350 cm^{-1} corresponding

to nitrile and NH_2 function, respectively. Its ^1H NMR spectrum showed two singlet signals at δ 2.32 and 7.35 due to CH_3 and 2NH_2 protons in addition to an aromatic multiplet in the region δ 7.38–7.61. Their mass spectra revealed in each case the respective molecular ion peak.

But, **3a–b** was condensed with 2-cyanoacetamide and 2-cyanothioacetamide in the presence of piperidine in ethanol to yield the bis pyridine carbonitrile derivatives **5a–c** in 60%, 35% and 70% yields, respectively and bis pyridnethione derivatives **6a,b** in 60% and 55% yields, respectively, as shown in Scheme 2. The structures of the newly synthesized compounds were confirmed based on their elemental analysis, IR, ^1H NMR and mass spectral data (see experimental). Compound **6a** was taken as an example reacted with each of 2-chloro-*N*-(4-bromophenyl) acetamide (**7a**), chloroacetamide (**7b**) by stirring in ethanolic sodium ethoxide solution at room temperature to give the corresponding 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis-6-(2-furyl)thieno[2,3-*b*]pyridine-2-carboxamide derivatives **9a,b**. However, treatment of the compound **6a** with hydrazine hydrate by refluxing in dioxane afforded the 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine] (**11**) as shown in Scheme 3. The structure of the products **9a,b** and **11** was elucidated by considering the data of IR, ^1H NMR, mass spectra and elemental analyses.

Scheme 3. 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis-6-(2-furyl)thieno[2,3-*b*]pyridine-2-carboxamide] derivatives **9a,b** and 4,4'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine] **11**.



3. Experimental Section

3.1. General Experimental Procedures

All melting points were measured on an Electrothermal Gallenkamp apparatus (Weiss-Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide discs on a Pye Unicam SP3300 and Shimadzo FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd. Cambridge, England and Shimadzu, Tokyo, Japan, respectively). The $^1\text{H-NMR}$ spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz). The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt. The biological evaluation of the products was carried out in the Microbiology Division of Micro-analytical Center of Cairo University. The starting material Pyrazole **1** was prepared as previously reported in the literature [39,41].

3.2. Synthetic Procedures

3.2.1. 1,1'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-Aryl prop-2-en-1-one] (**3a-c**)

A mixture of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**) (4.84 g, 20 mmol), the appropriate aldehyde **2a-c** (40 mmol) and 10% ethanolic sodium hydroxide solution (15 mL) in ethanol (30 mL) was stirred for 12 h. The reaction mixture was then warmed at 40 °C for 10 min. and the separated precipitate was filtered off and recrystallized from ethanol to afford the corresponding compounds **3a-c**.

3.2.2. 1,1'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-(2-furyl)prop-2-en-1-one] (**3a**)

Yield (65%), mp 180 °C (from EtOH); IR (KBr) ν_{max} : 2916, 2852 (aliphatic CH), 1658 (C=O), 1595 (C=C) cm^{-1} ; $^1\text{H NMR}$ (DMSO- d_6): δ 2.35 (s, 3H, CH₃), 6.60 (d, 1H, CH=, $J = 10.06$ Hz), 6.64 (d, 1H, CH=, $J = 6.4$ Hz), 7.05 (d, 1H, CH=, $J = 10.06$ Hz), 7.31 (d, 1H, CH=, $J = 9.4$ Hz), 7.26–7.89 (m, 11H, ArH). MS m/z (%): 398 (M^+ , 32.11), 277 (48.19), 265 (66.94), 230 (30.71), 213 (20.16), 154 (21.47). Anal. Calcd for C₂₄H₁₈N₂O₄ (398.41): C, 72.35; H, 4.55; N, 7.03. Found: C, 72.30; H, 4.52; N, 7.00%.

3.2.3. 1,1'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-(4-chlorophenyl) prop-2-en-1-one] (**3b**)

Yield (95%), mp 230 °C (from Dioxane); IR (KBr) ν_{max} : 3061 (aromatic CH), 2923 (aliphatic CH), 1663 (C=O), 1598 (C=C) cm^{-1} ; $^1\text{H NMR}$ (DMSO- d_6): δ 2.38 (s, 3H, CH₃), 7.24 (d, 1H, CH=, $J = 11.3$ Hz), 7.38 (d, 1H, CH=, $J = 10.5$ Hz), 7.54 (d, 1H, CH=, $J = 10.5$ Hz), 7.62 (d, 1H, CH=, $J = 11.3$ Hz), 7.66–7.84 (m, 13H, ArH). MS m/z (%): 490 ($\text{M}^+ + 3$, 4.86), 489 ($\text{M}^+ + 2$, 7.68), 488 ($\text{M}^+ + 1$, 23.38), 487 (M^+ , 14.66), 375 (7.36), 394 (11.66), 264 (0.96), 221 (0.33), 156 (2.20). Anal. Calcd for C₂₈H₂₀Cl₂N₂O₂ (487.37): C, 69.00; H, 4.14; N, 5.75; Cl, 14.55%. Found: C, 68.97; H, 4.12; N, 5.72; Cl, 14.53%.

3.2.4. 1,1'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-(4-methoxyphenyl)prop-2-en-1-one] (**3c**)

Yield (50%), mp 160 °C (from ethanol); IR (KBr) ν_{\max} : 3068 (aromatic CH), 2926, 2835 (aliphatic CH), 1661 (C=O), 1594 (C=C) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 2.22 (s, 3H, CH₃), 3.81 (s, 6H, 2OCH₃), 6.96 (d, 1H, CH=, J = 9.8 Hz), 7.26 (d, 1H, CH=, J = 10.7 Hz), 7.40 (d, 1H, CH=, J = 10.7 Hz), 7.50 (d, 1H, CH=, J = 9.8 Hz), 7.52–7.79 (m, 13H, ArH). MS m/z (%): 479 (M^+ + 1, 1.4), 478 (M^+ , 6.70), 371 (6.2), 345 (3.3), 317 (8.5), 264 (2.9), 212 (2.4), 156 (2.6). Anal. Calcd for C₃₀H₂₆N₂O₄ (478.53): C, 75.30; H, 5.48; N, 5.85%. Found: C, 75.28; H, 5.45; N, 5.84%.

3.2.5. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[2-amino-6-(aryl)nicotinonitrile] (**4a–c**)

Method A: A mixture of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**) (4.84 g, 20 mmol), malononitrile (2.64 g, 40 mmol), the appropriate aldehyde **2a–c** (40 mmol) and ammonium acetate (6.0 g), was heated under reflux in *n*-butanol (40 mL) for 3 h. On cooling, the separated yellow solid was filtered, washed with water and crystallized.

Method B: A mixture of each of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-Aryl prop-2-en-1-one] **3a–c** (1 mmol), malononitrile (2 mmol), and 0.616 g ammonium acetate (8 mmol) in *n*-butanol (40 mL) was refluxed for 3 h. After cooling, the precipitate was filtered off, dried, and crystallized to give **4a–c**.

3.2.6. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[2-amino-6-(2-furyl)nicotinonitrile] (**4a**)

Yield (50%), mp 230 °C (from ethanol); IR (KBr) ν_{\max} : 3438, 3376 (NH₂), 2203 (C≡N) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 2.39 (s, 3H, CH₃), 6.72 (s, 4H, 2NH₂), 7.11–7.96 (m, 13H, ArH). MS m/z (%): 526 (M^+ + 2, 3.87), 525 (M^+ + 1, 2.4214), 524 (M^+ , 100), 468 (45), 367 (7.51), 347 (2.48), 152 (3.05). Anal. Calcd for C₃₀H₂₀N₈O₂ (524.53): C, 68.69; H, 3.84; N, 21.36. Found: C, 68.67; H, 3.81; N, 21.35%.

3.2.7. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[2-amino-6-(4-chlorophenyl)nicotinonitrile] (**4b**)

Yield (40%), mp 210 °C (from ethanol); IR (KBr) ν_{\max} : 3438, 3350 (NH₂), 2210 (C≡N) cm^{-1} ; ^1H NMR (DMSO- d_6): δ : 2.32 (s, 3H, CH₃), 7.35 (s, 4H, 2NH₂), 7.38–7.61 (m, 15H, ArH). MS m/z (%): 615 (M^+ + 2, 6.6), 614 (M^+ + 1, 6.6), 613 (M^+ , 0.8), 456 (5.3), 384 (5.3), 153 (23.7), 118 (25). Anal. Calcd for C₃₄H₂₂Cl₂N₈ (613.49): C, 66.56; H, 3.61; N, 18.26; Cl, 11.56. Found: C, 66.54; H, 3.60; N, 18.25; Cl, 11.52%.

3.2.8. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[2-amino-6-(4-methoxyphenyl)nicotinonitrile] (**4c**)

Yield (45%); mp 200 °C (from ethanol); IR (KBr) ν_{\max} : 3456–3347 (NH₂), 2200 (C≡N) cm^{-1} ; ^1H -NMR (DMSO- d_6) (ppm): 2.47 (s, 3H, CH₃), 3.83 (s, 6H, 2OCH₃), 7.06 (s, 4H, 2NH₂), 7.33–7.64 (m, 15H, ArH's); ^{13}C -NMR: δ 30.4, 55.3, 84.6, 88.4, 104.2, 108.6, 109.6, 113.9, 114.3, 117.5, 121.7, 125.2, 126.7, 127.2, 128.2, 129.4, 129.9, 130.5, 133.2, 135.6, 139.2, 145.6, 146.5, 153.3, 155.6, 160.4, 160.9, 163.8; MS m/z (%): 604 (M^+ , 14), 347 (33.3), 257 (37), 119 (22.2). Anal. Calcd for C₃₆H₂₈N₈O₂ (604.68): C, 71.51; H, 4.67; N, 18.53. Found: C, 71.50; H, 4.65; N, 18.50%.

3.2.9. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-aryl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] (**5a–c**)

Method A: A mixture of each of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-aryl prop-2-en-1-one] **3a–c** (1 mmol), cyanoacetamide (0.17 g, 2 mmol), and (1.21 g, 16 mmol) ammonium acetate in *n*-butanol (40 mL) was refluxed for 3 h. After cooling, the precipitate was filtered off, dried, and crystallized to give **5a–c**.

Method B: A mixture of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**) (0.24 g, 1 mmol), appropriate aldehyde (2 mmol), cyanoacetamide (0.17 g, 2 mmol) and (1.21 g, 16 mmol) ammonium acetate in *n*-butanol (40 mL) was refluxed for 4 h. After cooling, the formed product was collected by filtration and crystallized to give **5a–c**.

3.2.10. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] (**5a**)

Yield (60%); mp 220 °C (from ethanol); IR (KBr) ν_{\max} : 3355 (NH), 2213 (C \equiv N), 1655 (C=O) cm^{-1} ; ^1H NMR (DMSO- d_6) (ppm): 2.35 (s, 3H, CH $_3$), 8.40 (s, 2H, 2NH), 6.91–7.51 (m, 13H, ArH); ^{13}C -NMR: δ 22.1, 90.4, 92.5, 95.0, 104.8, 110.4, 112.3, 114.1, 114.7, 115.6, 116.9, 123.1, 124.5, 129.5, 130.4, 137.8, 140.9, 142.0, 143.2, 143.9, 145.1, 146.7, 149.22, 152.3, 152.61, 153.2, 156.8; MS m/z (%): 527 ($\text{M}^+ + 1$, 1.19), 526 (M^+ , 2.26), 458 (0.02), 391 (3.08), 370 (16.12), 156 (7.36), 118 (41.54), 67 (13.06); Anal. Calcd for C $_{30}$ H $_{18}$ N $_6$ O $_4$ (526.52): C, 68.44; H, 3.45; N, 15.96. Found: C, 68.40; H, 3.41; N, 15.95.

3.2.11. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(4-chlorophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] (**5b**)

Yield (35%), mp 202 °C (from ethanol); IR (KBr) ν_{\max} : 3436 (NH), 2216 (C \equiv N), 1652 (C=O); ^1H NMR (DMSO- d_6): δ 2.37 (s, 3H, CH $_3$), 7.71 (s, 2H, 2NH), 7.25–7.51 (m, 15H, ArH). MS m/z (%): 615 (M^+ , 5.40), 385 (10.9), 154 (8.7), 119 (15.2). Anal. Calcd for C $_{34}$ H $_{20}$ Cl $_2$ N $_6$ O $_2$ (615.48): C, 66.35; H, 3.28; N, 13.65; Cl, 11.52. Found: C, 66.32; H, 3.25; N, 13.60; Cl, 11.50%.

3.2.12. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile] (**5c**)

Yield (70%), mp 190 °C (from ethanol); IR (KBr) ν_{\max} : 3437 (NH), 2216 (C \equiv N), 1664 (C=O) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 2.36 (s, 3H, CH $_3$), 3.83 (s, 6H, 2OCH $_3$), 7.65 (s, 2H, 2NH), 7.10–7.61 (m, 15H, ArH). MS m/z (%): 606 (M^+ , 1.9), 224 (3.7), 156 (3.7), 119 (18.1), 106 (4.2). Anal. Calcd for C $_{36}$ H $_{26}$ N $_6$ O $_4$ (606.65): C, 71.28; H, 4.32; N, 13.85. Found: C, 71.25; H, 4.30; N, 13.82%.

3.2.13. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile] (**6a,b**)

An equimolecular amount of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-(2-furyl)prop-2-en-1-one] (**3a**) (0.334 g, 1 mmol), 2-cyanoethanethioamide (0.2 g, 2 mmol) and few drops of

piperidine in Ethanol (30 mL) was heated under reflux for 5 h. After cooling, the solid product was collected by filtration, washed with ethanol and then recrystallized from ethanol to give **6a,b**.

3.2.14. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile] (**6a**)

Yield (60%), mp 260 °C (from ethanol); IR (KBr) ν_{\max} : 3425 (NH), 3053 (aromatic CH), 2924, 2853 (aliphatic CH), 2216 (C≡N) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 2.37 (s, 3H, CH₃), 7.98 (s, 2H, 2NH), 6.67–7.82 (m, 13H, ArH). MS m/z (%): 558 (M^+ , 1.2), 374 (81.8), 287 (22.7), 227 (50), 127 (22.7), 91 (59.1), 67 (22.7). Anal. Calcd for C₃₀H₁₈N₆O₂S₂ (558.64): C, 64.50; H, 3.25; N, 15.04; S, 11.48. Found: C, 64.48; H, 3.24; N, 15.00; S, 11.46%.

3.2.15. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile] (**6b**)

Yield (55%); mp 250 °C (from ethanol); IR (KBr) ν_{\max} : 3416 (NH), 3048 (aromatic CH), 2847 (aliphatic CH), 2206 (C≡N); ^1H NMR (DMSO- d_6) (ppm): δ 2.40 (s, 3H, CH₃), 8.00 (s, 2H, 2NH), 7.46–7.85 (m, 15H, ArH); ^{13}C -NMR: δ 21.5, 43.7, 94.3, 98.4, 102.3, 115.2, 120.8, 121.7, 124.1, 125.0, 128.2, 129.4, 130.1, 130.8, 131.6, 132.4, 133.5, 136.7, 139.7, 140.1, 142.6, 151.86, 157.5, 160.2, 161.8, 184.3, 188.0; MS m/z (%): 649 ($M^+ + 2$, 1.8), 648 ($M^+ + 1$, 2.2), 647 (M^+ , 3.5), 528 (11.6), 354 (11.6), 313 (23.3), 114 (20.9), 64 (100); Anal. Calcd for C₃₄H₂₀Cl₂N₆S₂ (647.61): C, 63.06; H, 3.11; N, 12.98; Cl, 10.95; S, 9.90. Found: C, 63.02; H, 3.10; N, 12.95; Cl, 10.93; S, 9.89%.

3.2.16. Synthesis of Compounds **9a,b**

General Procedure: A solution of each of **6a** (0.558 g, 1 mmol and 2-chloro-*N*-(4-bromophenyl)-acetamide (**7a**) (0.497 g, 2 mmol) or 2-chloroacetamide (**7b**) (0.187 g, 2 mmol) in sodium methoxide (prepared from 0.10 g of sodium and ethanol 25 mL) was stirred at room temperature for 15 min. The formed precipitate was collected by filtration, washed with water, ethanol and dried, to give **9a,b** respectively.

3.2.17. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-amino-*N*-(4-bromophenyl)-6-(2-furyl)thieno[2,3-*b*]pyridine-2-carboxamide] (**9a**)

Yield (50%); mp 180 °C (from ethanol); IR (KBr) ν_{\max} : 3475–3328, 3111 (NH and NH₂), 1641 (C=O) cm^{-1} ; ^1H -NMR (DMSO- d_6) (ppm): δ 2.34 (s, 3H, CH₃), 6.96 (s, 4H, 2NH₂), 7.21–8.06 (m, 21H, ArH), 9.55 (s, 2H, 2NH); ^{13}C -NMR: δ 23.7, 48.3, 105.1, 107.1, 109.2, 110.1, 111.2, 112.3, 112.5, 115.2, 120.9, 122.9, 125.2, 126.2, 127.3, 128.5, 129.3, 130.2, 131.1, 133.8, 137.3, 137.8, 138.2, 138.9, 139.6, 145.4, 147.4, 155.6, 157.4, 158.0, 159.0, 160.3, 161.5, 163.7, 165.2, 165.9; MS m/z (%): 984 ($M^+ + 2$, 33), 983 ($M^+ + 1$, 50), 982 (M^+ , 25), 953 (41), 783 (50), 588 (58), 156 (58); Anal. Calcd for C₄₆H₃₀Br₂N₈O₄S₂ (982.72): C, 56.22; H, 3.08; N, 11.40; S, 6.53; Br, 16.26. Found: C, 56.20; H, 3.00; N, 11.38; S, 6.50; Br, 16.23.

3.2.18. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[3-amino-6-(2-furyl) thieno[2,3-*b*]pyridine-2-carboxamide] (**9b**)

Yield (30%), mp 194 °C (from ethanol); IR (KBr) ν_{\max} : 3434, 3115 (NH and NH₂), 3062 (aromatic CH), 2953 (aliphatic CH), 1681 (C=O) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 2.40 (s, 3H, CH₃), 6.62–6.68 (s, 4H, 2NH₂), 7.13–7.87 (m, 17H, 2NH₂ and 13H ArH). MS *m/z* (%): 672 (M⁺, 34). Anal. Calcd for C₃₄H₂₄N₈O₄S₂ (672.75): C, 60.70; H, 3.60; N, 16.66; S, 9.53%. Found: C, 60.66; H, 3.59; N, 16.60; S, 9.50%.

3.2.19. 4,4'-(5-Methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis[6-(2-furyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine] (**11**)

A mixture of compound **6a** (5.58 g, 0.01 mol) and hydrazine hydrate (20–25 mL) was heated under reflux for 7 h. The solid product was filtered off, washed with EtOH, dried and crystallized from ethanol. Yield (35%), mp 265 °C (from ethanol); IR (KBr) ν_{\max} : 3314, 3194 (NH and NH₂), 2967 (aliphatic CH) cm⁻¹; ¹H NMR (DMSO-*d*₆): δ 2.49 (s, 3H, CH₃), 5.01 (s, 4H, 2NH₂), 6.66–7.99 (m, 13H, ArH), 8.66 (s, 2H, 2NH). MS *m/z* (%): 554 (M⁺, 0.2), 545 (9.4), 489 (26), 199 (15), 155 (15), 77 (100), 67 (11). Anal. Calcd for C₃₀H₂₂N₁₀O₂ (554.58): C, 64.97; H, 4.00; N, 25.26%. Found: C, 64.92; H, 3.98; N, 25.20%.

3.3. Antimicrobial Evaluation

The antibacterial and antifungal activity assays were carried out at the Microbiology Division of Microanalytical Center of Cairo University using the diffusion plate method [43–45]. A bottomless cylinder containing a measured quantity (1 mL, mg/mL) of the sample is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium, which has been heavily seeded with a spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism. The solvent used was DMSO and the concentration of the sample used is 100 µg/mL. The results of antimicrobial activity are summarized in Table 1. Most of the synthesized compounds were evaluated for their antibacterial against a Gram negative bacterium (*Escherichia coli* anaerobic (EC)), a Gram positive bacterium (*Staphylococcus aureus* (SA)) and for antifungal activity against *Candida albicans* (CA) and *Aspergillus flavus* (AF) by diffusion technique [43–45]. As seen from the data present in Table 1, *Escherichia coli* anaerobic is sensitive to compounds **3a**, **4c** and **9a**; furthermore, *Staphylococcus aureus* is sensitive to compounds **4b,c**, **5a** and **9a**. Whereas, all tested compounds did not exhibit the antifungal activity against the two tested fungi species *Candida albicans* and *Aspergillus flavus*. The activity of **4c** and **9a** is attributed to the presence of pharmacological active 4-methoxyphenyl at position 6 of the nicotinonitrile and 4-bromophenyl at position *N* of carboxamide.

Table 1. Antibacterial and antifungal activities of the synthesized compounds (3a–c, 4a–c, 5a,c, 6a,b) and 9a.

Compound No.	Inhibition Zone Diameter (cm)			
	Gram (–)	Gram (+)	Fungi	
	(EC)	(SA)	(AF)	(CA)
3a	10	0.0	0.0	0.0
3b	0.0	0.0	0.0	0.0
3c	0.0	0.0	0.0	0.0
4a	0.0	0.0	0.0	0.0
4b	0.0	12	0.0	0.0
4c	16	16	0.0	0.0
5a	0.0	10	0.0	0.0
5c	0.0	0.0	0.0	0.0
6a	0.0	0.0	0.0	0.0
6b	0.0	0.0	0.0	0.0
9a	13	14	0.0	0.0
Tetracycline	30	29		
Diflucan			18	21

ATCC for (EC, SA, SA and CA) are 11775, 12600 and 26555, respectively.

4. Conclusions

In conclusion, the reactivity of 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)diethanone (**1**) was investigated as a versatile and readily accessible building block for the synthesis of new bis-heterocycles incorporating 5-methyl-1-phenyl-1*H*-pyrazole moiety of biological and pharmaceutical importance.

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Conflict of Interest

The authors declare no conflict of interest.

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