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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME NOVEL PYRAZOLOPYRIDINE DERIVATIVES

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Eight pyrazolo[3,4-b]pyridine derivatives have been synthesized by Friedländer condensation of 5-aminopyrazole-4-carbaldehyde with active methylene compounds in basic medium. These compounds have been screened for their antibacterial activity against two Gram-negative and two Gram-positive bacterium. Pyrazolopyridines having the carboxamide group at the 5-position showed moderate to good activity against P. aeruginosa, E. coli, S. pneumoniae, and B. cereus.

Keywords: heterocycles, pyrazolo[3,4-*b*]pyridines, antimicrobial activity, disc diffusion method, Friedländer condensation.

The evolution of antibacterial resistance in bacterial strains against the currently available antibacterial agents is an increasing concern in recent years. For instance, Gram-positive bacterial pathogen such as *Staphylococcus aureus* is resistant to Methicillin, and *Streptococcus pneumoniae* and *Enterococci* are resistant to Penicillin and Vancomycin, respectively [1]. On the other hand, Gram-negative bacteria such as *H. influenza* and *M. catarrahalis* are resistant to β-lactams, quinolones, and macrolides [2]. In order to overcome the threat of widespread multidrug resistance in Gram-positive as well as Gram-negative bacterial strains, there is an ongoing demand for new antibacterials.

Pyrazolo[3,4-*b*]pyridines as aza-analogs of indazoles [3] have long served as attractive targets in organic synthesis due to their excellent antibacterial activity [4]. Moreover, pyrazolo[3,4-*b*]pyridines also exhibit other promising biological activities, including anxiolytic (e.g., Tracazolate) [5], dopamine D3-receptor antagonist and partial agonist [6], dopamine D4 antagonist [7], adenosine A1-receptor antagonist [8, 9], antiherpetic [10], anti-inflammatory [11], and antiallergic [12] properties. The methods so far reported for the synthesis of pyrazolopyridines include: i) the annelation of the pyrazole ring onto the suitably substituted pyridine ring [13-19], and ii) the annelation of the pyridine ring onto the appropriately substituted pyrazole ring [13, 20-30]. As part of our ongoing program [31] for the development of new antibacterials, we are particularly interested in the synthesis of pyrazolo[3,4-*b*]pyridines by the latter method. Like others, our strategy involves the Friedländer condensation of 5-aminopyrazoles with active methylene compounds as the key step. In this paper we report the full details of our efforts on the synthesis of some novel pyrazolo[3,4-*b*]pyridine derivatives and their antimicrobial profile against two Gram-positive and two Gram-negative bacterium species.

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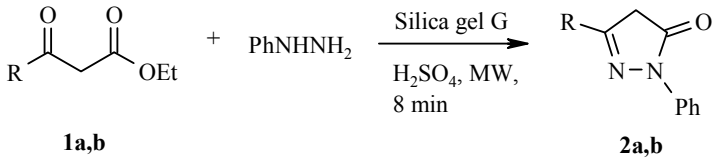
Our studies began with the pyrazolones **2a**,**b**, which were prepared by following a slight modificated procedure reported earlier [32]. The standard procedure for such pyrazolones includes the reaction of 1,3-dicarbonyl compounds with phenylhydrazine under reflux condition in ethanol. However, this reaction suffers from poor yield and long reaction time. In order to get a good yield of the product in a shorter reaction time, the reaction was carried out under microwave irradiation. Thus, treatment of equimolar amounts of 1,3-dicarbonyl compounds **1a**,**b** with phenylhydrazine and catalytic amounts (1-2 drops) of concentrated sulfuric acid adsorbed on silica gel G under microwave irradiation for 8 min gave pyrazolones **2a**,**b** in excellent yield (Scheme 1). This reaction procedure is remarkably simple and requires no solvent or inert atmosphere, and hence is an environmentally friendly process. It was also observed that when the reaction was carried out under microwave irradiation without silica gel, it gave a poor yield of the cyclic product.

Scheme 1

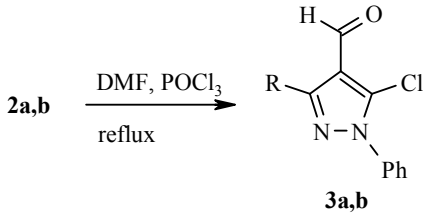


1a,b **2a,b**

1, 2 a R = Ph, **b** R = Me

The pyrazole derivatives **2a**,**b** were regioselectively formylated at C-4 using Vilsmeier–Haack reaction conditions (Scheme 2) to give compounds **3a**,**b** in moderate to good yield [33].

Scheme 2

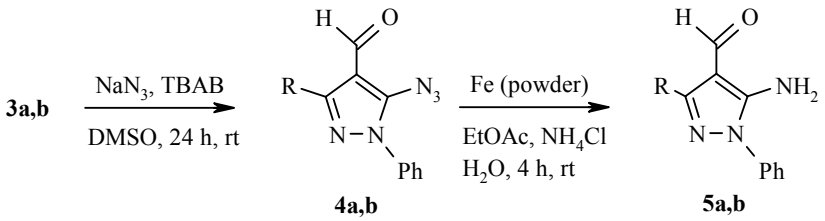


3a,b

3 **a** R = Ph, **b** R = Me

The chloro compounds **3a**,**b** were turned into the corresponding azides **4a**,**b** (Scheme 3) by the nucleophilic displacement of chlorine atom by azide anion, catalyzed by the phase-transfer agent tetrabutylammonium bromide. The formation of **4a**,**b** was evident from the spectral data. The IR spectra of compounds **4a,b** show a strong absorbance at 2148 cm^{-1} for the azide group. Chemoselective reduction of the azide group by iron powder in the presence of ammonium chloride [33] furnished *o*-amino aldehydes **5a,b** as pale-yellow crystalline solids in excellent yield.

Scheme 3

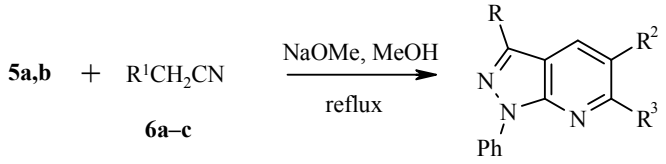


4a,b **5a,b**

TBAB – tetrabutylammonium bromide; **4, 5 a** R = Ph, **b** R = Me

The Friedländer condensation of *o*-amino aldehydes such as 5-aminopyrazole-4-carbaldehydes with ketones is described to take place either with strong bases or acids as catalysts; in special cases the ring closure can be observed without a catalyst at higher temperatures (e.g., under microwave irradiation) [34-37]. Having 5-aminopyrazolo-4-carbaldehyde in our hand, like others [4, 38–41] we have applied the Friedländer condensation reaction with active methylene compounds to get pyrazolo[3,4-*b*]pyridines. Initially we used piperidine [38] as the base for the condensation reaction, which unfortunately gave poor yield (< 20%). Then we tried other bases [29, 30, 42] like NaOH in EtOH, NaH in THF, and NaOMe in MeOH. We were pleased to find out that NaOMe catalyzed the condensation reaction to give the best yield.

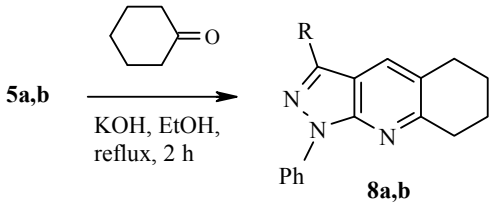
Scheme 4



6 a $R^1 = CONH_2$, **b** $R^1 = CN$, **c** $R^1 = CO_2Et$; **7 a**, **d**, **f** R = Me, **b**, **c**, **e** R = Ph, **a**, **b** $R^2 = CONH_2$, $R^3 = NH_2$, **c**, **d** $R^2 = CN$, $R^3 = NH_2$, **e**, **f** $R^2 = CONH_2$, $R^3 = OH$

Thus compounds **5a**,**b** on treatment with various active methylene compounds in the presence of an excess of sodium methoxide under reflux in methanol gave the corresponding pyrazolopyridines **7a-f** in good to excellent yields (Scheme 4). The amino aldehydes **5a**,**b** when reacting with active methylenes like ethyl cyanoacetate **6c** give amides **7e**,**f**, but with malononitrile **6b** the same reaction proceeds with the formation of nitriles **7c**,**d**. This may be due to the fact that the increased electronegativity of the oxygen atom in the OH group makes the adjacent nitrile group more reactive for conversion to amide. Formation of compound **7** was evident from spectral as well as analytical data. The reaction of compounds **5a**,**b** with cyclohexanone in the presence of KOH gave the corresponding annelated pyrazolopyridines **8a**,**b** [43] in good yield (Scheme 5). In order to test the biological activity of the synthesized pyrazolopyridines, compounds **7a**,**b** and **8a**,**b** were screened against two pathogenic Gram-positive and two Gram-negative bacteria and the results are shown in Table 1.

Scheme 5



8 a R = Ph, **b** R = Me

Antimicrobial Evaluation. The antimicrobial properties of the synthesized compounds were tested *in vitro* by the disc diffusion method [44, 45]. The antimicrobial properties are tested against two Gram-positive (e.g., *S. pneumoniae*, MTCC 1936, and *B. cereus*, MTCC 1305) and two Gram-negative (e.g., *P. aeruginosa,* NCIM 2074, and *E. coli*, NCIM 5051) pathogenic bacterial strains. The preliminary screening results indicated that compounds **7a**,**b** having the amino group at position 6 and the amide group at position 5 of the pyridine ring show pronounced biological activity. In compounds **7c**,**d**, although the amino group is present at position 6, the presence of the cyano group at position 5 diminishes the antibacterial activity. The presence of the OH group at

TABLE 1. Screening of Compounds Against Pathogenic Bacteria

Compound	Diameter of inhibition zone, mm / Concentration of compound, μg/disk											
	P. aeruginosa			*E. coli*			*S. pneumoniae*			*B. cereus*		
	60	80	100	60	80	100	60	80	100	60	80	100
7a	8	10	14	7	9	11	7	10	14	8	12	16
7b	9	11	16	—	—	—	—	—	—	12	14	15
7c	—	—	—	—	—	—	—	—	—	—	—	—
7d	—	—	—	—	—	—	—	—	—	—	—	—
7e	7	9	15	7	10	12	10	12	16	9	11	15
7f	8	10	14	—	—	—	9	11	16	10	12	16
8a	8	10	11	6	7	7	6	6	8	5	7	7
8b	8	10	11	6	7	7	6	6	8	7	8	10
Tetracycline (30 μg)		18			14			18			18	

position 6 along with the amide group at position 5 also confers similar inhibitory activity. Furthermore, compounds **8a**,**b** having no amide group at position 5 show somewhat lower antibacterial activity than compounds **7a**,**b**,**e**,**f**. From this observation it may be concluded that the presence of the cyano group at position 5 in the pyrazolo[3,4-*b*]pyridine ring is responsible for the loss of biological activity.

Conclusion. We have successfully synthesized a series of pyrazolo[3,4-*b*]pyridine *via* Friedländer condensation of 5-aminopyrazole-4-carbaldehyde with active methylene compounds. Compounds **7a**,**b**,**e**,**f** show significant antibacterial activity against four pathogenic bacterium species. The presence of the carboxamide group at position 5 of pyrazolo[3,4-*b*]pyridine may be responsible for pronounced antibacterial activity which, in turn, is suppressed by the cyano group in the same position.

EXPERIMENTAL

Reactions are generally performed under nitrogen atmosphere in flame-dried round bottom flasks. Reagents were purchased from suppliers like SRL, Spectrochem, Loba, s.d. Fine etc., and were used without further purification unless otherwise stated. Products were purified by column chromatography on silica gel (60–120 mesh, Rankem) or by recrystallization from distilled solvents. IR spectra were recorded on Perkin Elmer spectrophotometer on KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance Digital (400 and 100 MHz respectively) spectrometers using TMS as an internal standard in $CDCl_3$ (compounds **2a**,**b**, **3a**,**b**, **4a**,**b**, **5a**,**b**, **7c**,**d**, **8a**,**b**) and DMSO-d_6 (compounds **7a**,**b**,**e**,**f**). All ^{13}C spectra were proton decoupled; the "multiplicity" of the signals was determined using the DEPT technique. Mass spectra were taken by the EI (70 eV) technique on the Shimadzu QP 2010 PLUS GC-MS system. Elemental analyses were carried out with a Vario EL Elemental Analyzer. Melting points were uncorrected.

1,3-Diphenyl-1H-pyrazol-5(4H)-one (2a). To a mixture of ethyl benzoylacetate **1a** (0.45 ml, 2.6 mmol) and phenylhydrazine (0.25 ml, 2.6 mmol) a drop of concentrated sulfuric acid was added, and the resulting mixture along with 2 g of silica gel G was ground thoroughly. The mixture was heated in a domestic microwave oven (900 W) for 8 min. Then it was washed with CH_2Cl_2 repeatedly, the combined CH_2Cl_2 extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure yielding compound **2a** as a yellow crystalline solid, yield 92%; mp 110-128°C. IR spectrum, ν, cm^{-1}: 1709 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.01-7.22 (10H, m, two C_6H_5); 3.85 (2H, s, CH_2). ^{13}C NMR spectrum, δ, ppm: 170.26 (s); 154.67 (s); 138.13 (s); 130.87 (s, C=O); 130.74 (d); 128.94 (d); 128.89 (d); 126.00 (d); 125.33 (d); 119.09 (d); 39.67 (t, CH_2). Found, %: C 76.11; H 5.08; N 11.80; O 6.60. $C_{15}H_{12}N_2O$. Calculated, %: C 76.25; H 5.12; N 11.86; O 6.77.

3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one (2b). Ethyl acetoacetate (0.49 ml, 3.84 mmol) and phenylhydrazine (0.38 ml, 3.84 mmol) were taken, and compound **2b** was synthesized, following the same procedure as reported for **2a**, as a yellow solid, yield 90%; mp 112-122°C. IR spectrum, ν, cm^{-1}: 1598 (C=N). ^1H NMR spectrum, □, ppm: 7.88-7.15 (5H, m, C_6H_5); 3.44 (2H, s, CH_2); 2.21 (3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 170.57 (s, C=O); 156.27 (s); 138.04 (s); 128.83 (d); 125.05 (d); 118.89 (d); 43.11 (t, CH_2); 17.03 (q, 3-CH_3). Found, %: C 68.91; H 5.77; N 15.98; O 9.10. $C_{10}H_{10}N_2O$. Calculated, %: C 68.95; H 5.79; N 16.08; O 9.18.

5-Chloro-1,3-diphenyl-1H-pyrazole-4-carbaldehyde (3a). Compound **2a** (5.5 g, 23.5 mmol) was refluxed for 6 h with a mixture of $POCl_3$ (8.79 ml, 94.06 mmol) and N,N-dimethylformamide (7.27 ml, 94.06 mmol) and allowed to cool to room temperature. Then, cold water (80 ml) was added followed by neutralization with concentrated aqueous sodium hydroxide. The mixture was extracted with dichloromethane, and the organic layers were dried with anhydrous sodium sulfate and concentrated under reduced pressure. The crude solid was purified by column chromatography, yielding compound **3a** as white crystalline solid, yield 83%; mp 75°C. IR spectrum, ν, cm^{-1}: 1683 (C=O). ^1H NMR spectrum, δ, ppm (*J*, Hz): 10.01 (1H, s, CHO); 7.91-7.71 (2H, m, C_6H_5), 7.65-7.50 (2H, m, C_6H_5) 7.48–7.32 (6H, m, C_6H_5). ^{13}C NMR spectrum, δ, ppm: 183.94 (CHO); 154.30 (s); 136.99 (s); 133.26 (s); 130.79 (s); 129.61 (d); 129.49 (d); 129.33 (d); 128.97 (d); 128.61 (d); 125.50 (d); 116.46 (s).

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (3b). A mixture of compound **2b** (5 g, 28.73 mmol), DMF (7.11 ml, 91.95 mmol), and $POCl_3$ (18.79 ml, 201.11 mmol) was refluxed for 90 min. The reaction mixture was cooled to room temperature; cold water was added to it, followed by neutralization with aqueous sodium hydroxide. The mixture was extracted with dichloromethane, and the organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash-chromatography on silica gel to give compound **3b** as a white crystalline solid, yield 65%; mp 120°C. IR spectrum, ν, cm^{-1}: 1676 (C=O). ^1H NMR spectrum, δ, ppm: 10.00 (1H, s, CHO); 7.65-7.42 (5H, m, C_6H_5); 2.56 (3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 183.90 (d, CHO); 151.79 (s); 136.96 (s); 133.48 (s); 129.29 (d); 129.21 (d); 125.20 (d); 117.42 (s); 13.85 (q, 3-CH_3).

5-Azido-1,3-diphenyl-1H-pyrazole-4-carbaldehyde (4a). A mixture of compound **3a** (2.70 g, 9.82 mmol), sodium azide (0.77 g, 11.79 mmol), and tetrabutylammonium bromide (0.38 g, 1.17 mmol) was dissolved in DMSO (22 ml) and stirred at room temperature for 24 h. The reaction mixture was poured onto ice, and the solid precipitate was extracted with dichloromethane. The organic layer was washed repeatedly with water and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure to give compound **4a** as a cream-colored solid, yield 84%; mp 102–104°C. IR spectrum, ν, cm^{-1}: 2148 (N_3), 1666 (C=O). ^1H NMR spectrum, δ, ppm: 9.99 (1H, s, CHO); 7.82–7.62 (5H, m, C_6H_5); 7.58–7.42 (5H, m, C_6H_5). ^{13}C NMR spectrum, δ, ppm: 185.51 (d, CHO); 155.07 (s); 141.07 (s); 137.14 (s); 131.01 (d); 129.51 (d); 129.12 (d); 129.08 (d); 128.90 (d); 128.73 (d); 124.53 (s); 111.69 (s). Found, %: C 66.20; H 3.84; N 23.88; O 5.54. $C_{16}H_{11}N_5O$. Calculated, %: C 66.43; H 3.83; N 24.21; O 5.53.

5-Azido-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (4b). Compound **3b** (3.00 g, 13.61 mmol) was taken, and, following the same procedure as for compound **4a**, compound **4b** was synthesized in 95% yield; mp 42°C. IR spectrum, ν, cm^{-1}: 2144 (N_3), 1673 (C=O). ^1H NMR spectrum, δ, ppm: 9.93 (1H, s, CHO); 8.10-7.01 (5H, m, C_6H_5); 2.51(3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 183.62 (d, CHO); 152.07 (s); 137.02 (s); 129.11 (d); 128.56 (d); 124.28 (d); 112.33 (s); 12.72 (q, 3-CH_3). Found, %: C 57.90; H 4.06; N 30.55; O 7.09. $C_{11}H_9N_5O$. Calculated, %: C 58.14; H 3.99; N 30.82; O 7.04.

5-Amino-1,3-diphenyl-1H-pyrazole-4-carbaldehyde (5a). To a mixture of compound **4a** (2.76 g, 9.5 mmol), iron powder (1.72 g, 30.6 mmol), and ethyl acetate (10 ml), a solution of ammonium chloride (2.72 g, 50.9 mmol) in water (10 ml) was added, and the resulting emulsion was stirred at room temperature for 4 h. The mixture was filtered and the residue washed with ethyl acetate (2×10 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude solid was purified by column chromatography to give compound **5a** as a cream-coloured crystalline solid, yield 92%; mp 135-137°C. IR spectrum, ν, cm^{-1}: 3424, 3307 (NH_2), 1642 (C=O). ^1H NMR spectrum, δ, ppm: 9.90 (1H, s, CHO); 7.80-7.70 (2H, m, C_6H_5); 7.66-7.42 (8H, m,

C_6H_5); 6.00 (2H, br. s, NH_2). ^{13}C NMR spectrum, δ, ppm: 185.49 (d, CHO); 153.47 (s); 149.94 (s); 136.9 (s); 131.57 (s); 129.95 (d); 129.13 (d); 128.78 (d); 128.61 (d); 128.49 (d); 124.01 (d); 104.73 (s). Mass spectrum, *m/z*, (*I*, %): 263 $[M]^+$ (36), 77 $[M–C_{10}H_8N_3O]^+$ (100). Found, %: C 72.51; H 4.88; N 15.45; O 5.98. $C_{16}H_{13}N_3O$. Calculated, %: C 72.99; H 4.98; N 15.96; O 6.08.

5-Amino-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (5b). Compound **4b** (2.9 g, 12.9 mmol) was taken, and following the same procedure as for **5a**, compound **5b** was obtained as a yellow solid, yield 91%; mp 96°C. IR spectrum, ν, cm^{-1}: 3399, 3287 (NH_2), 1645 (C=O). ^1H NMR spectrum, δ, ppm (*J*, Hz): 9.64 (1H, s, CHO); 7.49-7.30 (5H, m, C_6H_5); 5.77 (2H, br. s, NH_2); 2.33 (3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 183.94 (d, CHO); 150.78 (s); 149.2 (s); 136.9 (s); 129.87 (d); 128.19 (d); 123.69 (d); 105.74 (s); 11.17 (q, 3-CH_3). Found, %: C 65.79; H 5.46; N 20.80; O 7.95. $C_{11}H_{11}N_3O$. Calculated, %: C 65.66; H 5.51; N 20.88; O 7.95.

6-Amino-3-methyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carboxamide (7a). To a solution of NaOMe (0.134 g, 2.487 mmol) in methanol, cyanoacetamide (0.049 g, 0.497 mmol) was added dropwise under an inert atmosphere. The resulting mixture was stirred for 0.5 h. A solution of compound **5b** (0.10 g, 0.49 mmol) dissolved in methanol was injected into the stirred mixture, which was then refluxed for 48 h, cooled to room temperature, neutralized by adding diluted HCl, and extracted with ethyl acetate. The organic layer was washed repeatedly with water, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography using methanol and diethylamine as eluent. Compound **7a** was obtained in 91% yield; mp 228°C. IR spectrum, ν, cm^{-1}: 3410, 3319 (NH_2), 1695 (C=O). ^1H NMR spectrum, δ, ppm: 8.52 (1H, s, H-4); 8.31-8.22 (2H, m, C_6H_5); 8.07 (1H, br. s, $CONH_2$); 7.78 (1H, br. s, $CONH_2$); 7.59-7.44 (2H, m, C_6H_5); 7.40 (2H, br. s, NH_2); 7.25-7.19 (1H, m, C_6H_5); 2.51 (3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 169.87 (s, $CONH_2$); 159.13 (s); 151.05 (s); 144.10 (s); 139.49 (s); 132.34 (d); 128.87 (d); 124.68 (d); 119.36 (d); 108.77 (s); 106.66 (s); 12.07 (q, 3-CH_3). Found, %: C 62.82; H 4.76; N 26.01; O 5.43. $C_{14}H_{13}N_5O$. Calculated, %: C 62.91; H 4.90; N 26.20; O 5.99.

6-Amino-1,3-diphenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carboxamide (7b) was obtained by Friedländer condensation of compound **5a** (0.1 g, 0.38 mmol) with cyanoacetamide in 65% yield; mp 234°C. IR spectrum, ν, cm^{-1}: 3412, 3330 (NH_2), 1665 (C=O). ^1H NMR spectrum, δ, ppm: 8.74 (1H, s, H-4); 8.41-8.28 (3H, m, C_6H_5); 8.14-8.08 (2H, m, C_6H_5); 7.85-7.75 (2H, br. s, $CONH_2$); 7.62-7.49 (6H, m, C_6H_5 + NH_2); 7.31 (1H, m, C_6H_5). ^{13}C NMR spectrum, δ, ppm: 169.82 (s, $CONH_2$); 158.90 (s); 151.60 (s); 144.86 (s); 139.27 (s); 132.86 (d); 132.11 (s); 128.96 (d); 128.92 (d); 128.84 (d); 127.12 (d); 125.44 (d); 120.29 (d); 108.20 (s); 106.49 (s). Mass spectrum, *m/z* (*I*, %): 329 $[M]^+$ (65), 311 $[M\text{-}NH_4]^+$ (46), 77 $[M\text{-}C_{13}H_{10}N_5O]^+$ (100). Found, %: C 69.07; H 4.33; N 21.22; O 4.74. $C_{19}H_{15}N_5O$. Calculated, %: C 69.29; H 4.59; N 21.26; O 4.86.

6-Amino-1,3-diphenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (7c). To a solution of sodium (0.8 g, 3.8 mmol) in dry methanol, malononitrile (0.04 ml, 0.76 mmol) was added dropwise under an inert atmosphere. The resulting mixture was stirred for 30 min. Compound **5b** (0.2 g, 0.76 mmol) dissolved in methanol was injected to the stirred mixture and the reaction mixture was refluxed for 8 h. The mixture was cooled to room temperature and neutralized by adding diluted HCl. The compound was extracted with chloroform, and the organic layer was washed with water several times. The organic layer was dried over Na_2SO_4 and concentrated. The crude compound was purified by column chromatography to give the target compound **7c** in 67% yield; mp 305°C. IR spectrum, ν, cm^{-1}: 2210 (CN), 3334, 3470 (NH_2). ^1H NMR spectrum, δ, ppm: 8.46 (1H, s, H-4); 8.28-8.22 (2H, m, C_6H_5); 7.98-7.90 (2H, m, C_6H_5); 7.60-7.45 (5H, m, C_6H_5); 7.40-7.32 (1H, m, C_6H_5); 5.45 (2H, br. s, NH_2). ^{13}C NMR spectrum, δ, ppm: 157.69 (s); 151.42 (s); 145.69 (s); 138.80 (s); 137.71 (d); 131.83 (s); 129.32 (d); 129.11 (d); 129.02 (d); 127.32 (d); 126.50 (d); 121.62 (d); 117.07 (s); 108.91 (s); 88.94 (s). Found, %: C 73.15; H 4.05; N 22.33. $C_{19}H_{13}N_5$. Calculated, %: C 73.30; H 4.21; N 22.49.

6-Amino-3-methyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (7d) was obtained by Friedländer condensation of compound **5b** (0.1 g, 0.497 mmol) with malononitrile in 63% yield; mp 225-226°C. IR spectrum, ν, cm^{-1}: 3469, 3333 (NH_2), 2209 (CN). ^1H NMR spectrum, δ, ppm: 8.10-7.95 (3H, m, H-4 and

C_6H_5); 7.45-7.30 (2H, m, C_6H_5); 7.25-7.12 (1H, m, C_6H_5); 5.31 (2H, br. s, NH_2); 2.46 (3H, s, 3-CH_3). ^{13}C NMR spectrum, δ, ppm: 157.93 (s); 150.95 (s); 144.08 (s); 138.80 (s); 136.49 (d); 128.99 (d); 126.10 (d); 121.15 (d); 117.20 (s); 110.66 (s); 87.72 (s); 12.35 (q, 3-CH_3). Found, %: C 67.34; H 4.38; N 28.03. $C_{14}H_{11}N_5$. Calculated, %: C 67.46; H 4.45; N 28.10.

6-Hydroxy-1,3-diphenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carboxamide (7e) was synthesized by Friedländer condensation of compound **5a** (0.1 g, 0.38 mmol) with ethyl cyanoacetate (0.04 ml, 0.38 mmol). Compound **7e** was purified by recrystallization from methanol, yield 95%; mp 280-282°C. IR spectrum, ν, cm^{-1}: 3457, 3354 (NH_2), 1688 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.81 (1H, s, H-4); 8.38–8.30 (2H, m, C_6H_5); 8.05-7.98 (2H, m, C_6H_5); 7.80 (2H, br. s, $CONH_2$); 7.60-7.28 (5H, m, C_6H_5); 7.25-7.12 (1H, m, C_6H_5). ^{13}C NMR spectrum, δ, ppm: 168.40 (s, $CONH_2$); 159.17 (s); 152.36 (s); 145.21 (s); 139.09 (s); 136.17 (d); 131.89 (d); 129.13 (d); 129.04 (d); 128.99 (d); 126.96 (d); 125.64 (d); 120.44 (s); 107.24 (s); 104.82 (s). Mass spectrum, *m/z* (*I*, %): 330 $[M]^+$ (78), 77 $[M\text{-}C_{13}H_9N_4O_2]^+$ (100). Found, %: C 68.97; H 4.11; N 16.71; O 9.74. $C_{19}H_{14}N_4O_2$. Calculated, %: C 69.08; H 4.27; N 16.96; O 9.69.

6-Hydroxy-3-methyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carboxamide (7f) was synthesized by Friedländer condensation of compound **5b** (0.05 ml, 0.497 mmol) with ethyl cyanoacetate (0.05 ml, 0.49 mmol), yield 63%; mp 270-276°C. IR spectrum, ν, cm^{-1}: 3410, 3313 (NH_2), 1698 (C=O). ^{1}H NMR spectrum, δ, ppm: 8.60 (1H, s, H-4); 8.30-8.20 (2H, m, C_6H_5); 7.77 (2H, br. s, $CONH_2$); 7.52-7.44 (2H, m, C_6H_5); 7.28-7.20 (1H, m, C_6H_5); 2.4 (3H, s, CH_3). ^{13}C NMR spectrum, δ, ppm: 168.57 (s, $CONH_2$); 159.39 (s); 151.83 (s); 144.75 (s); 139.32 (s); 135.82 (d); 128.88 (d); 124.86 (d); 119.54 (d); 109.50 (s); 103.38 (s); 12.04 (q, 3-CH_3). Mass spectrum, *m/z* (*I*, %): 268 $[M]^+$ (100), 250 $[M–H_2O]^+$ (35), 77 $[M\text{-}C_8H_7N_4O_2]^+$. Found, %: C 62.53; H 4.40; N 20.54; O 11.78. $C_{14}H_{12}N_4O_2$. Calculated, %: C 62.68; H 4.51; N 20.88; O 11.93.

1,3-Diphenyl-5,6,7,8-tetrahydro-1H-pyrazolo[3,4-*b*]quinoline (8a). A solution of compound **5a** (0.1 g, 0.38 mmol) and cyclohexanone (0.04 ml, 0.38 mmol) was refluxed in 2% KOH in ethanol for 2 h. Then ethanol was evaporated and to the residue water was added. After neutralization with dilute HCl the compound was extracted with dichloromethane. Recrystallization from ethanol gave the pure compound **8a** as yellow crystals, yield 86%; mp 138-142°C. IR spectrum, ν, cm^{-1}: 3040 (C–H), 2934, 2862 (CH_2). ^{1}H NMR spectrum, δ, ppm: 8.46-8.42 (2H, m, H-4, C_6H_5); 8.31-8.00 (3H, m, C_6H_5); 7.81-7.42 (5H, m, C_6H_5); 7.34-7.21 (1H, m, C_6H_5); 3.30-2.90 (4H, m, 5,8-CH_2); 2.22-1.80 (4H, m, 6,7-CH_2). ^{13}C NMR spectrum, δ, ppm: 158.42 (s); 150.07 (s); 143.48 (s); 139.88 (s); 133.17 (s); 130.07 (d); 128.97 (d); 128.89 (d); 128.48 (d); 127.27 (d); 127.16 (s); 125.48 (d); 120.95 (d); 114.18 (s); 33.60 (t, CH_2); 29.38 (t, CH_2); 23.09 (t, 6,7-CH_2). Found, %: C 81.07; H 5.69; N 12.66. $C_{22}H_{19}N_3$. Calculated, %: C 81.20; H 5.89; N 12.91.

3-Methyl-1-phenyl-5,6,7,8-tetrahydro-1H-pyrazolo[3,4-*b*]quinoline (8b) was synthesized by Friedländer condensation of compound **5b** (0.1 g, 0.49 mmol) with cyclohexanone (0.05 ml, 0.49 ml) as white crystals in 82% yield; mp 72-82°C. IR spectrum, ν, cm^{-1}: 3063 (C–H), 2935, 2862 (CH_2). ^{1}H NMR spectrum, δ, ppm: 8.50–8.21 (2H, m, H-4, C_6H_5); 7.88-7.68 (1H, m, C_6H_5); 7.65-7.42 (2H, m, C_6H_5); 7.40-7.12 (1H, m, C_6H_5); 3.35–2.82 (4H, m, 6,7-CH_2); 2.61 (3H, s, 3-CH_3); 2.26-1.80 (4H, m, 5,8-CH_2). ^{13}C NMR spectrum, δ, ppm: 158.22 (s); 149.29 (s); 129.27 (d); 128.97 (d); 126.05 (s); 125.09 (d); 120.51 (d); 115.93 (s); 33.51 (t, CH_2); 29.22 (t, CH_2); 23.07 (t, 6,7-CH_2); 12.46 (q, 3-CH_3). Found, %: C 77.43; H 6.32; N 15.65. $C_{17}H_{17}N_3$. Calculated, %: C 77.54; H 6.51; N 15.96.

Antimicrobial Screening. The antibacterial activity of the compounds was determined by the disc diffusion method [44, 45]. The bacteria were cultured in nutrient broth medium (from HiMedia) and used as inocula for the study. Bacterial cells were swabbed into nutrient agar medium in Petri dishes. The test solutions were prepared in DMSO to a concentration of 5 mg/ml, and then 12, 16, and 20 µl (60, 80, and 100 µg, respectively) of this solution were applied to filter paper discs (Whatmann No. 4, 5 mm dia), which were placed upon already seeded plates. These plates were incubated at 35±2°C for 24 h. Tetracycline was used as a standard positive control.

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