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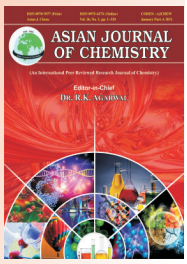

Pyrazolopyridines II: Synthesis and Antibacterial Screening of 6-Aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic Acids

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5-Amino-3-methyl-1-phenyl-1*H*-pyrazole was prepared to react with various aromatic aldehydes and pyruvic acid to afford 6-aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carbxylic acids. The prepared compounds were characterized by Mass, IR, ^{1}H, ^{13}C NMR spectra and CHN analysis data. Various 6-aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carbxylic acids synthesized during this work were also evaluated for their antibacterial activities. Some of these compounds were found to be good antibacterial agents.

Keywords: Pyrazolopyridines, Doebner Reaction, Schiff's bases, Pyruvic acid.

INTRODUCTION

Pyrazolopyridines are well known for their biological activities. These are found to be good antiviral agents, potent P-38 kinase and HIV reverse transcriptases[1-4]. A number of pyrazolopyridines have also been reported as good vasodilating, anti inflammatory, analgesics, antipyretic, antimalarial and antibacterial compounds[5-11]. We have already presented the synthesis of some novel ethyl 1,6-diaryl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylates[12] which may be considered as ester analogs of cincophen. In continuation of our work, we would now like to report our success with their corresponding 4-carboxylic acids and their antibacterial screening.

EXPERIMENTAL

The chemicals used were commercially available from Merck or Fluka and were used as such. However when needed were purified using normal techniques. The solvents used were distilled and dried. FTIR spectra were recorded on Bruker Tensor-27. Melting points were taken on a GallenKamp melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were taken on Bruker DPX instrument at 400 MHz and 100 MHz, respectively.

Preparation of 6-Aryl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids (2a-2h) (Table-1)

General procedure: A mixture of equimolar amounts of respective 5-aminopyrazole (2.0 mmol), appropriate aromatic aldehydes (2 mmol) and one drop of HCl was heated without any solvent at 100 °C on an oil bath. It was followed by successive addition of an equimolar amount of pyruvic acid and 3 mL of glacial acetic acid. The mixture was further heated under reflux for 2 h, allowed to cool to room temperature, filtered and dried. The crude compounds were recrystallized from ethanol.

In the case of solid aldehydes (products **2c** and **2f**), the Schiff bases were prepared by heating the mixture under reflux in ethanol (5 mL). Ethanol was evaporated and followed by the same general procedure as described above.

1,6-Diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid. (2a): Yield: 35 %; m.p.: 270-274 °C. lit.[11]

TABLE-1
6-ARYL-3-METHYL-1-PHENYL-1*H*-PYRAZOLO[3,4-b]PYRIDINE-4-CARBOXYLIC ACIDS (**2a-2h**)

Compound	X	Yield (%)	m.p. (°C)
2a	H	35	272-274*
2b	2-Cl	46	214-216
2c	4-Cl	47	294**
2d	3-CH_3	38	176-178
2e	4-OC_2H_5	34	192-194
2f	3-NO_2	29	210-212
2g	4-F	46	266-268
2h	4-OH	40	260-262

*lit.[11] mp:285-287; ** lit.[11] m.p.:280-282

285-287 °C; IR (KBr, ν_{max}, cm^{-1}): 3170 (O-H); 3060-2950 (C-H); 1714 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.81 (s, 3H, CH_3), 7.35-8.36 (m, 11H, ArH), 13.81 (br.s, 1H, COOH); ^{13}C NMR ($CDCl_3$): δ 15.89 (CH_3), 112.00, 115.46, 121.48, 126.57, 127.43, 128.82, 128.93, 129.64, 134.90, 138.32, 139.08, 142.92, 152.02, 156.85, 167.41 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{15}N_3O_2$) [M^+]: 329.12; Found: 329.0 Anal. Calcd. for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76. Found: C, 72.54; H, 5.09; N, 13.07.

6-(3'-Chlorophenyl)-3-methyl-1-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-4-carboxylic acid. (2b): Yield: 45 %; m.p.: 214-216 °C; IR (KBr, ν_{max}, cm^{-1}): 3152 (O-H); 3030-2920 (C-H); 1705 (C=O); 1585 (C=C); ^{1}H NMR (DMSO): δ 2.70 (s, 3H, CH_3), 7.3-8.3 (m, 10H, ArH); ^{13}C NMR (DMSO): δ 15.96 (CH_3), 115.31, 121.47, 125.55, 125.93, 127.49, 128.96, 129.66, 130.06, 134.88, 135.01, 139.01, 140.09, 142.90, 155.15, 167.16 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{14}N_3O_2Cl$) [M^+]: 363.08 & [M + 2]: 365.08; Found: 363.0 & 365.1; Anal. Calcd. for $C_{20}H_{14}N_3O_2Cl$: C, 66.03; H, 3.88; N, 11.55. Found: C, 65.74; H, 3.78; N, 11.34.

6-(4'-Chlorophenyl)-3-methyl-1-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-4-carboxylic acid. (2c): Yield: 47 %; m.p.: 294 °C lit.[11] 280-282 °C; IR (KBr, ν_{max}, cm^{-1}): 3148 (O-H); 3055-2940 (C-H); 1702 (C=O); 1582 (C=C); ^{1}H NMR (DMSO); δ 2.70 (s, 3H, CH_3), 7.30-8.4 (m, 9H, ArH), 8.18 (s, 1H, H-5); ^{13}C NMR (DMSO): δ 15.56 (CH_3), 111.71, 114.37, 120.77, 125.86, 129.05, 129.17, 134.98, 136.19, 136.34, 138.73,142.28, 151.24, 154.78, 166.41 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{14}N_3O_2Cl$) [M^+]: 363.08 & [M + 2]: 365.08; Found: 363.1 & 365.1; Anal. Calcd. for $C_{20}H_{14}N_3O_2Cl$: C, 66.03; H, 3.88; N, 11.55. Found: C, 66.03; H, 3.74; N, 11.60.

3-Methyl-1-phenyl-6-*m*-tolyl-1*H*-pyrazolo[3,4-*b*]-pyridine-4-carboxylic acid. (2d) : Yield: 38 %; m.p.: 176-178 °C; IR (KBr, ν_{max}, cm^{-1}): 3120 (O-H); 3030-2920 (C-H); 1715 (C=O); ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, $PhCH_3$), 2.82 (s, 3H, CH_3), 7.26-8.35 (m, 9H, ArH), 8.22 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$): δ 16.43 (CH_3), 21.63 ($ArCH_3$), 111.97, 116.62, 124.65, 125.87, 126.84, 128.91, 129.01, 129.85, 130.79, 132.88, 138.16, 138.60, 139.30, 142.64, 152.21, 156.99, 169.45 (C=O); MS: *m/z* (%) = Calcd. for ($C_{21}H_{17}N_3O_2$) [M^+]: 343.13; Found: 343.2; Anal. Calcd. for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.69; H, 5.24; N, 11.78.

6-(4'-Ethoxyphenyl)-3-methyl-1-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-4-carboxylic acid. (2e): Yield: 34 %; m.p.:192-194 °C; IR (KBr, ν_{max}, cm^{-1}): 3220 (O-H); 3040-2930 (C-H); 1713 (C=O); 1572 (C=C); ^{1}H NMR (DMSO): δ 1.45 (t, 3H, OCH_2CH_3, *J* = 6.8), 2.80 (s, 3H, CH_3), 4.11 (q, 2H, OCH_2CH_3, *J* = 6.8), 7.0-8.35 (m, 10H, ArH); ^{13}C NMR (DMSO): δ 14.38 (OCH_2CH_3), 15.58 (CH_3), 63.45 (OCH_2), 111.22, 114.57, 121.56, 124.93, 125.93, 128.93, 129.82, 130.50, 134.69, 138.95, 142.79, 151.89, 156.52, 160.38, 167.32 (C=O); MS: *m/z* (%) = Calcd. for ($C_{22}H_{19}N_3O_3$) [M^+]: 373.14; Found: 373.1; Anal. Calcd. for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.31; H, 5.52; N, 11.16.

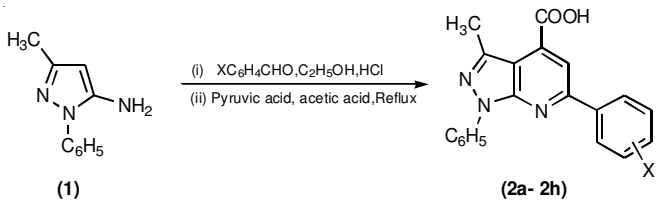
3-Methyl-6-(3'-nitrophenyl)-1-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-4-carboxylic acid. (2f): Yield: 29 %; m.p.: 210-212 °C; IR (KBr, ν_{max}, cm^{-1}): 3130 (O-H); 3050-2930 (C-H); 1700 (C=O); 1596 (C=C), 1525, 1345 (NO_2); ^{1}H NMR (DMSO): δ 2.71 (s, 3H, CH_3), 7.3-9.0 (m, 10H, ArH); ^{13}C NMR (DMSO): δ 15.52 (CH_3), 112.22, 114.70, 120.70, 120.97, 121.76, 124.5, 126.11, 129.04, 129.19, 130.70, 133.59, 138.59, 139.10, 142.38 148.49, 151.11, 153.59, 166.34 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{14}N_4O_4$) [M^+]: 374.10; Found: 374.0; HRMS (EI): Calcd. for ($C_{20}H_{14}N_4O_4$) [M^+]: 374.1671 ; Found: 374.1626.

6-(4'-Fluorophenyl)-3-methyl-1-phenyl-1*H*-pyrazolo [3,4-*b*]pyridine-4-carboxylic acid. (2g): Yield: 46 %; m.p.: 266-268 °C; IR (KBr, ν_{max}, cm^{-1}): 3160 (O-H); 3060-2950 (C-H); 1710 (C=O); 1593 (C=C); ^{1}H NMR (DMSO): δ 2.70 (s, 3H, CH_3), 7.30-8.35 (m, 9H, ArH), 8.15 (s, 1H, H-5); ^{13}C NMR (DMSO): δ 15.58 (CH_3), 111.48, 114.31, 115.86, 116.08, 120.75, 125.84, 129.18, 129.60, 129.69, 134.05, 136.18, 138.79, 142.27, 151.27, 155.04, 162.19, 164.66, 166.49 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{14}N_3O_2F$) [M^+]: 347.11; Found: 347.1; HRMS (EI): Calcd. for ($C_{20}H_{14}N_3O_2F$) [M^+]: 347.1072 ; Found: 347.1097.

6-(4'-Hydroxyphenyl)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acid. (2h): Yield: 40 %; m.p.: 260-262 °C; IR (KBr, ν_{max}, cm^{-1}): 3298 (O-H); 3025-2940 (C-H); 1706 (C=O); 1594 (C=C); 3298 (OH); ^{1}H NMR (DMSO): δ 2.70 (s, 3H, CH_3), 6.95-8.35 (m, 9H, ArH), 8.05 (s, 1H, H-5), 9.98 (br. s, 1H, OH); ^{13}C NMR (DMSO): δ 15.58 (CH_3), 110.77, 113.66, 115.86, 120.59, 125.56, 128.39, 128.90, 129.15, 135.93, 138.97, 142.21, 151.47, 156.25, 159.57, 166.68 (C=O); MS: *m/z* (%) = Calcd. for ($C_{20}H_{15}N_3O_3$) [M^+]: 345.11; Found: 345.11; Anal. Calcd. for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.38; N, 12.17. Found: C, 69.29; H, 4.59; N, 12.08.

RESULTS AND DISCUSSION

6-Aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids (**2a-2h**) were prepared according to **Scheme-I**, from 5-amino-3-methyl-1-phenyl-1*H*-pyrazole[13]. 5-Aminopyrazoles were prepared to react with various aromatic aldehydes to obtain respective Schiff's bases which were, without isolation, subjected to condensation with pyruvic acid to give 3-methyl-1-phenyl-6-aryl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carboxylic acids (**2a-2h**). The yields of the recrystallized products are in the range of 29 to 47 %; the crude yields are although much higher. No attempts, at this stage, were made to optimize them. All the products obtained as fine crystals, are stable compounds at room temperature and were characterized by IR, ^{1}H, ^{13}C NMR spectra.



Scheme-I

Spectral characterization of synthesized compounds: In all these compounds C=O group of acid functionality at 4th position showed an absorption band in the IR spectral range of 1715-1700 cm^{-1}. In the ^{1}H NMR spectra a singlet in the range of 2.70 to 2.80 ppm confirmed the presence of three proton of methyl group present at position 3 of pyrazole ring.

TABLE-2
ANTIBACTERIAL ACTIVITY OF 6-ARYL-3-METHYL-1-PHENYL-1*H*-PYRAZOLO[3,4-b]PYRIDINE-4-CARBOXYLIC ACIDS

Compound	*B. subtilis* (+) MIC_{50}	*S.aureus* (+) MIC_{50}	*S.typhi* (-) MIC_{50}	*P.aureginosa* (-) MIC_{50}	*E.coli* (-) MIC_{50}	*S. sonnei* (-) MIC_{50}
2a	8.41 ± 0.18	16.78 ± 0.22	13.04 ± 0.33	12.99 ± 0.24	16.85 ± 0.00	7.65 ± 0.11
2b	18.17 ± 0.17	15.42 ± 0.15	11.05 ± 0.32	11.73 ± 0.28	-	7.39 ± 0.77
2c	19.04 ± 0.51	-	12.27 ± 0.35	13.07 ± 0.12	-	16.03 ± 0.11
2d	13.56 ± 0.50	18.62 ± 0.29	12.88 ± 0.13	14.31 ± 0.22	16.03 ± 0.46	13.91 ± 0.17
2e	-	19.21 ± 0.28	14.38 ± 0.00	12.02 ± 0.29	-	10.91 ± 0.32
2f	14.69 ± 0.33	13.58 ± 0.21	15.33 ± 0.23	14.55 ± 0.01	-	13.72 ± 0.41
2g	-	7.65 ± 0.21	13.26 ± 0.41	12.01 ± 0.21	18.17 ± 0.50	10.98 ± 0.23
2h	-	-	-	-	-	-
Ampicilin	11.66 ± 0.14	10.69 ± 0.06	10.85 ± 0.16	12.33 ± 0.15	11.32 ± 0.13	11.98 ± 0.13
Gentamycin	10.36 ± 0.13	8.42 ± 0.12	11.21 ± 0.31	10.89 ± 0.11	8.21 ± 0.02	9.31 ± 0.18
Ciprofloxacin	8.36 ± 0.12	9.42 ± 0.11	7.59 ± 0.11	11.03 ± 0.10	8.21 ± 0.02	7.31 ± 0.08

The H-5 of the pyridine ring appeared as a singlet in the range of 8.15 to 8.22 ppm.The carbonyl carbon in all ^{13}C NMR spectra gave a discrete signal in the range of 166.34 to 167.45 ppm. CHN analysis and HRMS also confirmed the structure of the prepared compounds.

Antibacterial studies

Procedure: The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments[14,15]. The test samples with suitable solvents and dilutions were pipetted into wells (20 µg/well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37 °C for 16-24 h with lid on the microplate. The absorbance was measured at 540 nm using microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. Ciprofloxacin, gentamycin and ampicilin were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/well) and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software and data expressed as MIC_{50} (µg/mL). Results are mean of triplicate (n = 3, ± sem).

Table-2 shows the results of MIC_{50} studies of 6-aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-4-carbxylic acids (**2a**-**2h**) against six strains. Compound **2a** showed comparable activity against the *B. subtilis* (+) and *S. sonnei* (-) to the standard ciprofloxacin. Compound **2b** was found to show lower MIC_{50} value (MIC_{50} = 11.05 ± 0.32) against *S. typhi* (-) than the gentamycin (MIC_{50} = 10.85 ± 0.16). It is also found to be more active against *P. aureginosa* (-) (MIC_{50} = 11.73 ± 0.28) than ampicilin (MIC_{50} = 12.33 ± 0.15). However it has a comparable MIC_{50b}value (MIC_{50} = 7.39 ± 0.77) to the ciprofloxacin (MIC_{50} = 7.31 ± 0.08) against *S. sonnei* (-). Compound **2e** is a little more active (MIC_{50} = 12.02 ± 0.29) than ampicilin (MIC_{50} = 12.33 ± 0.15) against *P. aureginosa* (-). Compound **2g** showed some significant results. It is found to be more active against *S. aureus* (+) (MIC_{50} = 7.65 ± 0.21) than gentamycin (MIC_{50} = 8.42 ± 0.12). It also showed lower MIC_{50} value against *P. aureginosa* (-) (MIC_{50} = 12.01 ± 0.21) than ampicilin (MIC_{50} = 12.33 ± 0.15). Moreover it is found to be more active against *S. sonnei* (-) (MIC_{50} = 10.98 ± 0.23) than ampicilin (MIC_{50} = 11.98 ± 0.13). So we can assume that this series of compound showed significant antibacterial activity.

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