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Synthesis and Study of Antibacterial Effect of 3,4 -Diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile

Kailas Rangnath Labhade
Department of Chemistry, S.V.K.T. Arts, Science and Commerce College, Deolali Camp, Nashik, Maharashtra, INDIA.

ABSTRACT

Objectives: The present study was focused on environment friendly synthesis of cynopyridone compounds. New improved, three component, one-pot, solvent-free method was successfully developed for the synthesis of 3,4 -diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile using ammonium acetate as a green catalyst. **Materials and Methods:** Use of multicomponent, one pot synthetic approach in organic synthesis is becoming popular due their simple procedure and high yield of the product. This approach requires less time and minimum raw materials as compared to stepwise synthetic methods. Aromatic aldehyde, ethyl cyanoacetate and 3-aryl-1-phenyl-1*H*-pyrazol-5-amine (or 1,3-diphenyl-1*H*-pyrazol-5-amine) in presence of ammonium acetate at elevated temperature produce title compound in good yield. Structure determination of newly synthesized compounds were done by using IR and NMR spectroscopy. Furthermore, compounds were screened for their *in vitro* antibacterial activity towards gram-positive (*B. subtilis, S. aureus*) and gram-negative (*K. pneumoniae, E. coli*) bacteria by using the disc diffusion method. **Results:** The results of the antibacterial study showed that many substituted cynopyridone compounds have good to moderate antibacterial activity. Compounds having two halogen substituents showed better activity as compare to other compounds. **Conclusion:** This new improved method offers many advantages like easy workup, mild reaction conditions, good quality product and improvement in product yield as compared to earlier methods. By using this multi-component approach, we can significantly reduce the production cost and environmental pollution.

Keywords: Ammonium Acetate, Antibacterial activity, Green catalyst, One-pot synthesis, Pyrazolo[3,4-b]pyridine.

Correspondence

Dr. Kailas R Labhade
Department of Chemistry, S.V.K.T. Arts, Science and Commerce College, Deolali Camp, Nashik, Savitribai Phule Pune University, Pune, Maharashtra, INDIA.

Email id: kailaslabhade@gmail.com

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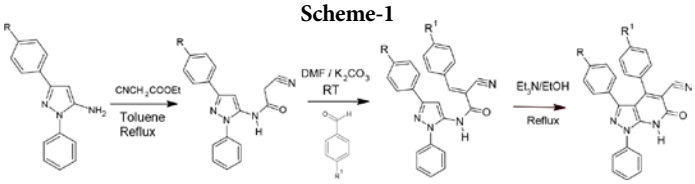
INTRODUCTION

The biological activities of cynopyridone compounds are well known and show various pharmacological activities like antifungal, antiviral, antibacterial, antihypertensive, antioxidant, and antitumor.[1-8] Apart from this, 3-pyridine carbonitriles have been studied for their fluorescence properties and these compounds are utilized in the synthesis of optical fibres, information display, security paper, dyes etc.[9-11] Due to the presence of nitrile and carbonyl groups, these compounds were extensively used for the synthesis of various heterocyclic compounds and various methods have been reported for their synthesis.[12-16] Abbas Rahmati,[17] Mahdavinia GH[18] and Khatri TT[19] reported the Synthesis of cyanopyridone compounds by using a multi-component approach. While ammonium acetate catalysed synthesis was reported by Yehya M. Elkholy,[20] MH Khan[21] and Shridhar Malladi.[22] In our earlier work, we have successfully synthesized, the new 3,4 -diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile by multistep reaction sequence [Scheme 1], as well as one-pot synthesis.[15] In the stepwise synthetic method overall yield of the product is decreased due to a greater number of steps involved in the synthesis. By using the multicomponent method, we have successfully increased the yield of the product and also reduced the production cost.[15] Inspired by our previous work, we have planned to develop a new environment-friendly improved method for the synthesis of these compounds having low production cost.

Scheme-1



MATERIALS AND METHODS

Experimental

All A.R. grade chemicals were used without further purification. Melting points were recorded by using digital melting point apparatus. IR spectra were recorded on Shimadzu IR-408, (Shimadzu FTIR instrument) by using KBr and values were expressed in cm^{-1}. The ^{1}H or ^{13}C spectral analysis was carried out by using dimethyl sulfoxide (DMSO-d6) and deuterated chloroform ($CDCl_3$) solvent and were recorded on NMR spectrometer. TMS is used as an internal standard and chemical shifts are expressed in δ (ppm).

General procedure

Synthesis of 3,4-diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*] pyridine-5-carbonitrile (4).

In 25 ml round bottom flask, 3-aryl-1-phenyl-1*H*-pyrazol-5-amine 1 (or 1,3-diphenyl-1*H*-pyrazol-5-amine) (1mmol), ethyl cyanoacetate 2 (1 mmol), aromatic aldehyde 3 (1mmol) and 40 mole percent ammonium acetate were added. The water condenser was attached, and the reaction mixture was heated at 130°C in an oil bath for 30 min (TLC check). After cooling, 50 ml distilled water was added and the reaction mixture was stirred for 30 min. The precipitate obtained after filtration was washed

with distilled water. The dried product was recrystallised by using a suitable solvent.

Antibacterial Study

In vitro Antibacterial study of 3,4 -diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5 carbonitrile (4) was carried out against *B. subtilis and S. aureus* (gram -positive) and K. *pneumoniae and E. coli* (gram negative*)* bacteria by conventional disk diffusion agar method.[23-24] Sterile nutrients agar butts were inoculated with pure cultures and pour plate technique was performed. Sterile filter paper disc impregnated with solution of synthesized compound prepare in DMSO, were placed in inoculated plates. Concentrations used were 50 and 100 ug/ml. Broad spectrum antibiotic ciprofloxacin was used as standard drug. The plates were further incubated at 37°C for 24 hrs and zone of inhibition was recorded (Table 3).

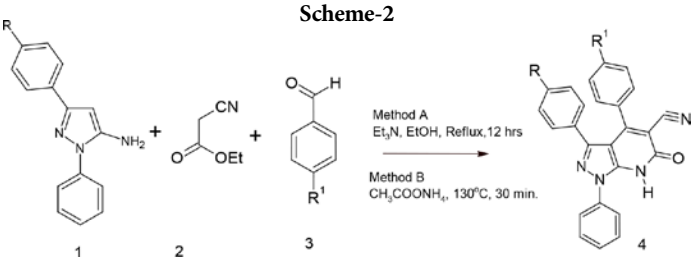
4-(4-chlorophenyl)-6-oxo-1,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4j)

White solid, Yield: 95%, m.p. 204-206^{0}C; IR (KBr): 3353, 3270 (NH), 1660 (C=O), 2218 (CN), 1600(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.39-7.43(m, 4H, Ar-H), δ 7.48 (d, *J=8.50 Hz*, 2 H, Ar-H), δ 7.51 (d, *J=8.60 Hz*, 2 H, Ar-H), δ 7.82 -7.84 (m,4H Ar-H), δ 7.87 (d, *J=8.40, Hz*, 2 H, Ar-H), δ, 13.21 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 92.99, 115.97, 124.17, 126.87, 127.63, 128.40, 128.66, 129.58, 132.04, 133.49, 138.52, 139.58,141.39,145.93, 150.40, 151.92, 157.90 160.04.

4-(4-bromophenyl)-6-oxo-1,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4k)

White solid, Yield: 94%, m.p. 210-212°C; IR (KBr): 3360, 3265 (NH), 1658 (C=O), 2220 (CN), 1610(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.38-7.42(m, 3H, Ar-H), δ 7.49-7.52(m, 1H, Ar-H), δ 7.57-7.63(m, 4H, Ar-H), δ 7.70 (d, *J=8.50 Hz*, 1H, Ar-H), δ 7.85 (d, *J=8.50 Hz*, 2 H, Ar-H), δ 7.91 (d, *J=8.50 Hz*, 1H, Ar-H), δ 8.04 (d, 2H Ar-H), δ, 13.30 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 95.76, 116.76, 124.55, 127.05, 128.83, 129.04, 129.80, 130.19, 130.27, 131.19, 131.41, 134.03, 136.04,137.34, 142.10, 144.93, 151.66, 157.10,160.10

Scheme-2



4-(4-methylphenyl)-6-oxo-1,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile(4l)

White solid, Yield: 92%, m.p. 198-200°C; IR (KBr): 3365, 3260 (NH), 1660 (C=O), 2218(CN), 1600(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.44(s, 3H, CH_3),), δ 7.32 (d, *J=8.60 Hz*, 2H, Ar-H),), δ 7.39 (d, *J=8.50 Hz*, 2H, Ar-H), δ 7.48-7.51(m, 1H, Ar-H), δ 7.57-7.62(m, 4H, Ar-H), δ 7.82-7.87(m, 4H, Ar-H), δ, 13.20 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 21.90, 93.70, 115.72, 124.50, 126.10, 127.30, 128.20, 129.10, 130.18, 130.78, 132.29, 132.90, 134.20, 136.22,145.32, 147.90, 150.60, 156.20, 158.62.

3-(4-chlorophenyl)-6-oxo-1,4-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4m)

White solid, Yield: 94%; m.p. 210-212°C; IR (KBr): 3350, 3275 (NH), 1660 (C=O), 2220 (CN), 1600(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.37-7.41(m, 4H, Ar-H), δ 7.50 (d, *J=8.50 Hz*, 2 H, Ar-H), δ 7.58 (d, *J=8.60 Hz*, 2 H, Ar-H), δ 7.84 -7.87(m,4H Ar-H),δ 7.90(d, *J=8.40, Hz*, 2 H, Ar-H), δ, 13.10 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 93.20, 114.56, 125.40, 126.70, 127.96, 128.60, 129.80, 130.60, 131.12, 132.40, 134.20, 137.13, 139.24,140.59,145.58, 149.40, 151.30, 157.20, 159.10.

3-(4-bromophenyl)-6-oxo-1,4-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4n)

White solid, Yield: 94%, m.p. 214-216°C; IR (KBr): 3358, 3270 (NH), 1660 (C=O), 2218 (CN), 1600(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.40-7.45(m, 3H, Ar-H), δ 7.48-7.52(m, 1H, Ar-H), δ 7.60-7.64(m, 4H, Ar-H), δ 7.71 (d, *J=8.50 Hz*, 1H, Ar-H), δ 7.84 (d, *J=8.50 Hz*, 2 H, Ar-H), δ 7.90 (d, *J=8.50 Hz*, 1H, Ar-H), δ 8.03(d, 2H Ar-H), δ, 13.24 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 95.60, 115.66, 124.40, 126.30, 127.10, 128.90, 130.80, 131.39, 131.32, 133.56, 134.24, 136.06,137.40, 145.25, 150.10, 153.68, 177.90, 159.30.132.67,

3-(4-methylphenyl)-6-oxo-1,4-diphenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4o)

White solid, Yield: 96%, m.p. 194-196°C; IR (KBr): 3360, 3265 (NH), 1660 (C=O), 2220(CN), 1600(C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.42(s,

Entry	R	R′	Product	Yield [a] (%) Method A	Yield [b] (%) Method A	Yield [b] (%) Method B
1	Cl	Cl	4a	85	-	94
2	Cl	Br	4b	84	-	95
3	Cl	CH_3	4c	80	-	92
4	Br	Cl	4d	84	-	94
5	Br	Br	4e	85	-	94
6	Br	CH_3	4f	82	-	92
7	CH_3	Cl	4g	87	-	95
8	CH_3	Br	4h	86	-	94
9	CH_3	CH_3	4i	88	-	95
10	H	Cl	4j	-	80	95
11	H	Br	4k	-	78	94
12	H	CH_3	4l	-	82	92
13	Cl	H	4m	-	80	94
14	Br	H	4n	-	82	94
15	CH_3	H	4o	-	84	96

a=reported yield as per literature 15 b=isolated yield

3H, CH_3),), δ 7.320 (d, *J=8.60 Hz*, 2H, Ar-H),), δ 7.40(d, *J=8.50 Hz*, 2H, Ar-H), δ 7.45-7.48(m, 1H, Ar-H), δ 7.58-7.63(m, 4H, Ar-H), δ 7.80-7.85(m, 4H, Ar-H), δ, 13.30 (br. s, 1H, -NH) ; ^{13}C NMR ($CDCl_3$): δ 22.10, 94.50, 115.72, 123.40, 124.10, 126.33, 127.30, 128.90, 131.20, 131.78, 133.20, 134.33, 134.90, 135.40,144.22, 146.40, 151.24, 158.20, 160.20.

RESULTS

Synthetic route for the desired, 3,4 -Diaryl-6-oxo-1-phenyl-6,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (4) is presented in Scheme 2. Various catalyst like p-toluene sulphonic acid, triethylamine, zinc chloride, piperidine and ammonium acetate were tried for the screening. Out of these triethylamine and ammonium acetate were found to have better catalytic effect. (Table 1).

Optimisation of reaction condition for ammonium acetate catalyst was studied by changing catalyst concentration and temperature and it was found that ammonium acetate with 40 mole percent concentration and at 130°C temperature gave the best results (Table 2). By using this approach, we have successfully developed a new improved, three component, one-pot, solvent-free method for synthesis of compound 4 having 92% to 96 % yields.

Antibacterial study showed that majority of cynopyridone derivatives have good to moderate antibacterial activity. Compounds 4a, 4b, 4d, 4e showed better activity as compared to other compounds (Table 3).

DISCUSSION

The model reaction in this synthesis was three component, one-pot reaction involving 1,3-diphenyl-1*H*-pyrazol-5-amine), 4-Chlorobenzaldehyde and ethyl cyanoacetate. (Scheme 2). Initially, we concentrate our attention on the use of different catalysts. Various catalyst like p-toluene sulphonic acid, triethylamine, zinc chloride, piperidine and ammonium acetate were tried for the screening. Out of these triethylamine and ammonium acetate were found to have better catalytic activity. In our earlier study, we have employed triethylamine in a three-component reaction,[15] hence change in catalyst concentration, change of solvent and variation in reaction conditions were tried for this catalyst, but no significant improvement was observed in the yield of the product. The reaction catalysed by ammonium acetate showed significant improvement and we have tried different solvent and various temperature condition for optimisation of the reaction. To find the required concentration of catalyst and suitable heating temperature for model reaction, various concentration of ammonium acetate and different heating temperatures were tried. (Table 1). The results showed that good quality and greater yield of product 4 was obtained by using 40 mole percent catalyst at 130°C temperature. For the higher concentration of catalyst (more than 40 mole percent) no further increment in product yield was observed. Under similar reaction conditions, different 5-amino pyrazole (1) and various aromatic aldehyde (2) having activating and deactivating groups were used. All reactions proceeded smoothly and produced products in good yield. Some synthesized compounds (4a-i) are known and reported earlier by us, identification of these compounds was done by comparing physical properties and spectral data with the literature.[15] Other new products (4j-o) were characterized by infrared spectra, ^{1}H NMR and, ^{13}C NMR spectra. *In vitro* antibacterial study was carried out by using Ciprofloxacin as standard antibiotic drug and DMSO as a solvent. It was observed that there was no effect of DMSO on these bacteria. Outcome of the antibacterial study was presented in Table 3, and it was observed that majority of cynopyridone derivatives have good to moderate antibacterial activity. Introduction of halogen in title compound showed significant enhancement in antibacterial effect. Compounds (4a, 4b, 4d, 4e) having two halogen substituents showed better activity as compare to other compounds

Table 1: Effect of Catalyst, catalyst concentration, solvent and temperature on % yield of the product.

Entry	Catalyst	Mole %	Solvent	Temperature	Time (hours)	Yield (%)
1	CH_3COONH_4	10	-	130°C	0.5	88
2	CH_3COONH_4	20	-	130°C	0.5	90
3	CH_3COONH_4	30	-	130°C	0.5	92
4	CH_3COONH_4	40	-	130°C	0.5	95
5	CH_3COONH_4	50	-	130°C	0.5	95
6	CH_3COONH_4	50	Ethanol	Reflux	12	60
7	Et_3N	20	Ethanol	Reflux	12	78
8	Et_3N	40	Ethanol	Reflux	12	80

Reaction conditions: 1,3-diphenyl-1H-pyrazol-5-amine 1(1 mmol), Ehtyl cyano acetate 2 (1mmol), 4-Chlorobenzaldehyde 3 (1 mmol).

Table 2: Optimization of the Reaction Conditions.

Entry	Catalyst	Catalyst Mole %	Solvent	Condition	Time (hours)	Yield
1	Et_3N	40	Ethanol	Reflux	12	80
2	Et_3N	40	Methanol	Reflux	12	52
3	CH_3COONH_4	40	Ethanol	Reflux	12	60
4	CH_3COONH_4	40	Methanol	Reflux	12	50
5	CH_3COONH_4	40	-	90°C	0.5	82
6	CH_3COONH_4	40	-	110°C	0.5	90
7	CH_3COONH_4	40	-	130°C	0.5	95

Reaction conditions: 1,3-diphenyl-1H-pyrazol-5-amine 1(1 mmol), Ehtyl cyano acetate 2 (1 mmol) and 4-Chlorobenzaldehyde 3 (1 mmol)

Table 3: Antibacterial activities of 3,4 -diaryl-6-oxo-1-phenyl-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile.

Compound	Concentration µg /ml	Antibacterial Activity Zone of Inhibition (mm)			
		Gram Positive Bacteria		Gram Negative Bacteria	
		Bacillus subtilis	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*
4a	50	10	09	09	10
	100	15	12	14	15
4b	50	08	08	09	09
	100	14	13	15	15
4c	50	-	-	-	-
	100	8	09	09	7
4d	50	07	06	07	07
	100	13	12	14	12
4e	50	08	06	06	06
	100	14	13	15	13
4f	50	-	-	-	-
	100	09	8	07	9
4g	50	-	-	-	-
	100	08	09	7	10
4h	50	-	-	-	-
	100	08	08	09	07
4j	50	-	-	-	-
	100	10	07	09	8
4k	50	-	-	-	-
	100	08	11	08	09
4m	50	-	-	-	-
	100	10	09	08	08
4n	50	-	-	-	-
	100	08	09	07	08
Ciprofloxacin	50	20	18	21	21
	100	28	25	30	29

CONCLUSION

We have developed an environment friendly, simple and improved method for synthesis of 3, 4 diaryl-6-oxo-1-phenyl-6, 7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile by using ammonium acetate as a green catalyst. By using this multi-component approach, we had significantly reduced the production cost and environmental pollution. This new method offers many advantages like easy workup, mild reaction conditions, good quality product and improvement in product yield as compared to earlier methods. The result of the antibacterial study showed that many compounds have good to moderate antibacterial activity.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

IR: Infrared; **FTIR:** Fourier-transform infrared; **KBr:** Potassium bromide; **DMSO:** Dimethyl Sulfoxide; **$CDCl_3$:** Deuterated Chloroform; **NMR:** Nuclear Magnetic Resonance; **TMS:** Tetramethyl silane; **mmol:** millimole; **TLC:** Thin layer chromatography; **m.p.:** Melting point; **µg / ml:** Micrograms per millilitre.

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