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SYNTHESIS OF SOME PYRAZOLO (3,4-b) PYRIDINE DERIVATIVES PROVEN EFFECTIVE AS ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Archana D. Jadhav[a*], Pankaj Barhate[a], Ayesha Durrani[b]

a. Department of Chemistry, Loknete Ramdas Patil Dhumal Arts, Science & Commerce College Rahuri, Ahmednagar, Maharashtra, India
b. Department of Chemistry, Dr. Rafiq Zakaria College of Womens, Naukhanda, Aurangabad, Maharashtra, India
Email: archu1990jadhav@gmail.com

ABSTRACT
In recent years heterocyclic compounds have more importance because of their pharmacological activities. 5-aminopyrazole derivatives have a particular value due to their broad spectrum of biological activity and their wide ranging utility as synthetic tools in the design of various bioactive molecules. The synthesis of pyrazolo (3,4-b) pyridines can be achieved by condensation of 1,3-diketone such as acetyl acetone with 5-amino pyrazole by conventional and microwave irradiation method. The structure of newly synthesized compounds was characterized on the basis of IR and NMR, analysis. The newly synthesized compounds were screened for antibacterial and antifungal activity.

KEY WORDS
Antibacterial, irradiation, NMR, acetonitrile, antifungal, etc.

INTRODUCTION
Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and also called as azoles [1]. Recently pyrazole derivatives have been found in nature as β-(1-pyrazolyl) alanine was isolated from the seeds of water melons [1]. Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial [2], antiviral [3], antitumor [4-5], antidepressant [6], insecticides and fungicides [7]. Several pyrazole derivatives have been found to possess significant activities such as antiproliferative [8], antiparasitic [9], herbicides [10]. A number of pyrazole have also been reported to have interesting biological activities like anti-inflammatory [11] and antiprotozoal [12-13] which render them valuable active ingredients of medicine and plant protecting agents.

Present work deals with the preparation of some pyrazolo (3,4-b) pyridine derivative which was prepared by using 3-(aryl)-1-phenyl-1H-pyrazol-5-amine and 1,3-diketone via

condensation reaction by using conventional and microwave irradiation methods. The structures of newly synthesized compounds are characterized on the basis of IR and NMR, analysis. The newly synthesized compounds were screened for their antibacterial and antifungal activities. In this work on the advances of derivatives of pyrazole has some biological activities are observed [14]. Working with such types of pyrazole derivatives shows some important properties like simplifying criteria, minimum time requirement and role of importance in biological study [15-16]. Many attempts have been made to synthesize, characterize and study to biological activity of pyrazole derivatives [17].

Combinatorial chemistry as a new method for the rapid generation of a great number of structurally diverse substances, being required widely a great impact of drug discovery [18-19]. In recent experiment we have tried to decrease the reaction time required to complete the reaction for that purpose we had successfully driven our reaction. These derivatives have acquired versatile importance as drug substances in pharmaceutical industry. In this work the investigation of novel derivatives formation we use easily available starting materials and their broad range of antifungal & antibacterial activity was evaluated.

MATERIAL AND METHODS

All the chemicals and solvents were obtained from E-Merck and SD fine chemicals L.T.D. India (AR, LR grade). Melting points were determined in open capillaries in liquid paraffin bath and are uncorrected. Purity of the compound was routinely checked on silica gel TLC plates using $CHCl_3$ and sometimes CCl_4 as solvent. ^1H NMR spectra were recorded on Bruker AV, 200 MHz spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scale. Multiplicities of ^1H NMR signals are designated as s (singlet), d (doublet), dd (doublet of doublet), dt (doublet of triplet), t (triplet), quin (quintet), m (multiplet) etc. IR data were recorded an Alpha-T ATR-FTIR.

PRESENT WORK (Synthesis of pyrazolo (3,4-b) pyridine derivatives)

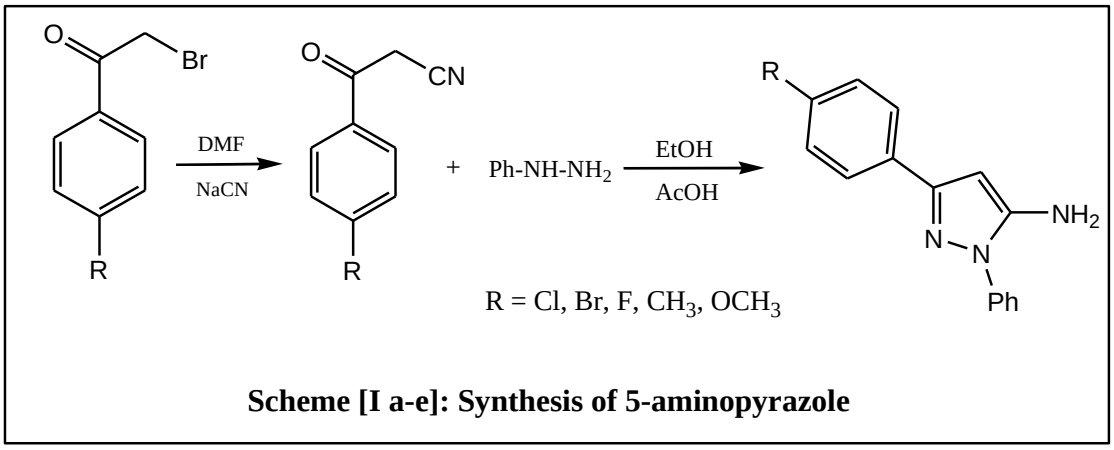
A) Conventional method:

The synthesis of Pyrazolo (3,4-b)pyridines, was successfully achieved by cyclocondensation reaction of 5-aminopyrazole a-e (0.01 mol) with acetyl acetone (0.02 mol) in acidic condition. The reaction mixture was refluxed for 10-12 hr. furnished the desired yellow coloured solid with 66-67% yield. (TLC checked)

B) Microwave method:

When same reaction mixture was irradiated in microwave at 150 ^0C for 15-20 min. furnished yellow coloured solid. It was observed that the compound obtained by both method are one and the same, it was characterized by spectral and analytical techniques.

The required5-aminopyrazole was synthesized by starting with benzoylacetonitrile. In our work we used SN^2 displacement of substituted phenacyl bromide or phenacyl chloride with sodium cyanide, yielded required benzoylacetonitrile in good yield[20-21].



Scheme [I a-e]: Synthesis of 5-aminopyrazole

1) Synthesis of pyrazolo [3,4-b]pyridine derivatives using 1,3-diketone (II a-e):

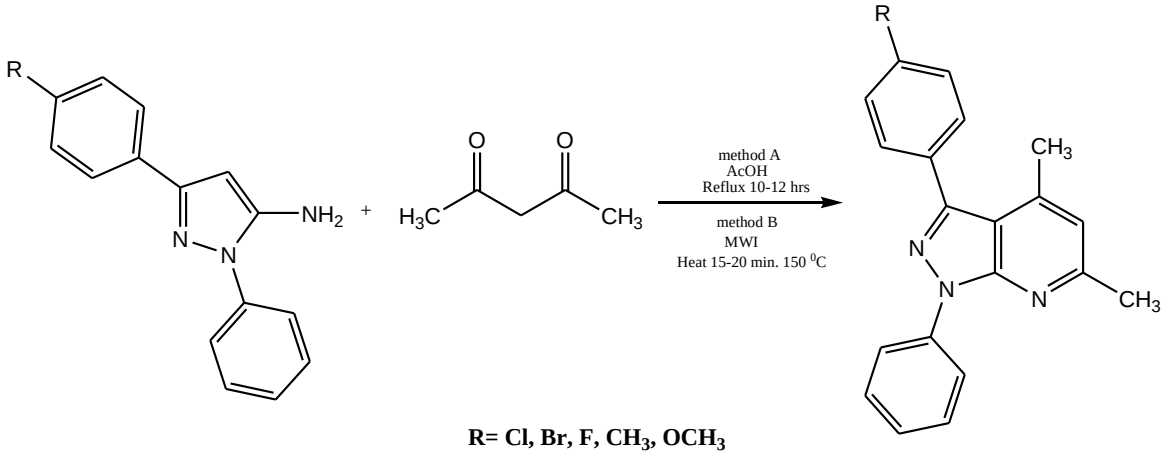
5-amino pyrazole (I a-e) react with acetyl acetone to obtained (II a-e) by conventional and microwave methods below,

A: Conventional method:

The synthesis of pyrazolo[3,4-b]pyridines, (II a-e) was successfully achieved in cyclo-condensation reaction of 5-aminopyrazole (I a-e) (0.27 g, 1 mmol) with acetyl acetone (0.1 ml, 1mmol) in acetic acid (25 ml). The reaction mixture was refluxed for 10-12 hours. The solid separated on cooling, was filtered, dried and crystallized from methanol to yield (II a-e).

B: Microwave method:

A mixture of 5-aminopyrazoles (I a-e) (0.27 g, 1mmol) and acetyl acetone (0.1 ml, 1 mmol) was irradiated in microwave at 150 ^{0}C for 15-20 min. The solid separated on cooling was filtered, dried and crystallized from methanol to yield (II a-e).



Scheme [II a-e]: Synthesis of pyrazolo[3,4-b]pyridine derivatives using 1,3-diketone

TABLE 1

Parameters	A: Conventional method					B: Microwave method			
	Time (hrs.)	**Yield (%)**	**Melting point (^{0}C)**	**Solvent used**	**Amount of solvent (ml)**	**Time (hrs.)**	**Yield (%)**	**Melting point (^{0}C)**	**Solvent used**
a	10-12	68	166-168	Acetic acid	25	1	83	166-168	-
b	10-12	66	188-190	Acetic acid	25	1	84	188-190	-
c	10-12	68	170-172	Acetic acid	25	1	82	170-172	-
d	10-12	66	176-178	Acetic acid	25	1	82	176-178	-
e	10-12	68	184-186	Acetic acid	25	1	84	184-186	-

ANTIBACTERIAL ACTIVITY

The plates were inoculated by specific microorganism by spread plate technique; bores were made in the solidified agar plate by using a sterile borer. The test solution of specified concentration was added in the bore by using sterile pipette and the plates were kept in freeze for 1 hour for diffusion and then incubated at 37 ^{0}C for 24 hours. After 24 hours the plates were examined and zone of inhibition were recorded. All the synthesized compounds were screened for antibacterial activity against both gram positive S. aureus & Bacillus substilis and gram negative E. coli & Proteus vulgaris bacteria at a concentration of 100 µg/ml, 200 µg/ml, 400µg/ml, 800 µg/ml. Ampicillin Capsules was used as standard for comparison of antibacterial activity of samples. In presence of base such as NaOH and ethanol is used as a solvent. The result of screening is given below.

TABLE 2: Zone of inhibition for Gram positive bacteria

Compound (II a-e)	Zone of inhibition (mm)							
	Staphylococcus aureus				**Bacillus subtilis**			
	100 µg	**200 µg**	**400 µg**	**800 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**
a	08	09	12	15	09	10	13	16
b	07	09	11	13	08	09	11	12
c	09	10	13	15	09	12	14	17
Ampicillin	10	12	14	17	10	13	15	18

TABLE 3: Zone of inhibition for Gram negative bacteria

Compound (II a-e)	**Zone of inhibition (mm)**							
	Escherichia coli				**Proteus vulgaris**			
	100 µg	**200 µg**	**400 µg**	**800 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**
a	08	09	10	12	08	10	11	13
b	07	08	10	11	07	08	10	12
c	08	10	12	14	09	11	12	14
Ampicillin	09	11	13	15	10	12	14	16

Amongst all synthesized compounds II (a) & II(c) were found to be more potent as antibacterial *Staphylococcus aureus, Bacillus subtilis*agents and *Escherichia coli, Proteus vulgaris* as agents. The zone of inhibition of synthesized compounds was compared with the standard drug Ampicillin at four different concentrations.

ANTIFUNGAL ACTIVITY

The antifungal testing was carried out against *Aspergillusniger& Candida ablicans,* known antifungal drug Itraconazole as an standard. The zone of inhibition measured in mm. amongst all these synthesized compound shows significant activity.

TABLE 4: Zone of inhibition for antifungal activity

Compound (II a-e)	**Antifungal activity Zone of inhibition (mm)**	
	Aspergillusniger	**Candida ablicans**
a	13.2	11.4
b	10.6	10.8
c	14.2	11.7
Itraconazole	15.6	12.4

PHYSICAL, ELEMENTAL AND SPECTRAL ANALYSIS

1) 3-(4-chorophenyl)-4,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-b]pyridine II (a)

M. P.; 166-168 ^{0}C; **Yield:** 68 % & 87 %; **IR (KBr):** 2338, 1578, 1503 cm^{-1} ;

^{1}H NMR (CDCl$_3$): δ (ppm):2.42 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.87 (s, 1H, ArH), 7.29 (t, J = 7.8 Hz, 1H, ArH), 7.44-7.53 (m, 4H, ArH), 7.59 (d, J = 8.4 Hz, 2H, ArH), 8.30 (d, J = 8.4 Hz, 2H, ArH); **^{13}C NMR (CDCl$_3$) δ (ppm):** 27.2, 32.3, 120.2, 128 (2C'S), 130.2, 132.4, 135.2 (2C'S), 137.3, 140.1, 141 (2C'S), 142.2, 149.3 (2C'S), 152.1, 154.2, 156.7, 163.3.

2) 3-(4-bromophenyl)-4,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-b]pyridine II (b)

M. P.; 188-190 ^{0}C; **Yield:** 66 % & 84 %; **IR;** 2334, 1578, 1506 cm^{-1};

^{1}H NMR (CDCl$_3$): δ (ppm) ; 2,41 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.87 (s, 1H, ArH), 7.28 (t, J = 7.8 Hz, 1H, ArH), 7.47-7.53 (m, 4H, ArH), 7.61 (d, J = 8.4 Hz, 2H, ArH), 8.30 (d, J = 8.4 Hz, 2H, ArH); **^{13}C NMR (CDCl$_3$): δ (ppm) ;** 26.7, 33.1, 120.4, 128.4 (2C'S), 130.6, 132.2, 136.2 (2C'S), 137.9, 140.6, 141.3 (2C'S), 143.5, 149.9 (2C'S), 152.4, 155.9, 157.3, 162.8.

3) 3-(4-fluorophenyl)-4,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine II (c)
M. P.; 170-172 ^{0}C; **Yield:** 68 % & 82 % ; **IR:** 2334, 1573, 1506;
^{1}H NMR ($CDCl_3$): δ (ppm) ; 2.44 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.87 (s, 1H, ArH), 7.28 (t, J = 7.8 Hz, 1H, ArH), 7.48-7.55 (m, 4H, ArH), 7.63 (d, J = 8.4 Hz, 2H, ArH), 8.30 (d, J = 8.4 Hz, 2H, ArH); **^{13}C NMR ($CDCl_3$): δ (ppm) ;** 24.1, 32.5, 120.6, 128.2 (2C'S), 131.5, 132.8, 134.5 (2C'S), 138.0, 140.8, 142.8 (2C'S), 144.3, 150 (2C'S), 153.8, 160, 154.3, 161.2.

4) 4,6-dimethyl-1-phenyl-3-p-tolyl-1H-pyrazolo[3,4-b]pyridine II (d)
M.P.; 176-178 ^{0}C; **Yield:** 66 % & 82 % ; **IR:** 2351, 1580, 1506 cm^{-1};
^{1}H NMR ($CDCl_3$): δ (ppm) ; 2.44 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.87 (s, 1H, ArH), 7.28 (t, J = 7.8 Hz, 1H, ArH), 7.48-7.55 (m, 4H, ArH), 7.63 (d, J = 8.4 Hz, 2H, ArH), 8.30 (d, J = 8.4 Hz, 2H, ArH); **^{13}C NMR ($CDCl_3$): δ (ppm) ;** 18.7, 25.1, 31.3, 121.6, 127.3 (2C'S), 130.4, 133.5, 137.4 (2C'S), 138.6, 141.8, 143.5 (2C'S), 145.3, 151.3 (2C'S), 157.8, 159.4, 156.8, 160.4

5) 3-(4-methoxyphenyl)-4,6-dimethyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine II (e)
M. P.; 185-187 ^{0}C; **Yield:** 68 % & 84 %; **IR:** 2355,1582, 1505 cm^{-1};
^{1}H NMR ($CDCl_3$): δ (ppm) ; 2.44 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.87 (s, 1H, ArH), 7.28 (t, J = 7.8 Hz, 1H, ArH), 7.48-7.55 (m, 4H, ArH), 7.63 (d, J = 8.4 Hz, 2H, ArH), 8.30 (d, J = 8.4 Hz, 2H, ArH); **^{13}C NMR ($CDCl_3$): δ (ppm) ;** 18.3, 23.5, 30.6, 121.4, 126.8 (2C'S), 131.2, 132.8, 136.5 (2C'S), 138.4, 140.8, 145.2 (2C'S), 146.3, 151.1 (2C'S), 154.3, 158.4, 160.8 and 161.7

CONCLUSION

The main target of our reaction is to reduce the reaction time and efficiency of the product. Here, we have presented an operationally simple, suitable, fast, efficient method for the preparation of Pyrazole derivative. The main focus of this research work was to synthesize, purify, characterize and evaluate antibacterial & antifungal activities of the synthesized compounds & which shows good antibacterial as well as antifungal activity.

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