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An efficient synthesis of 1, 4- disubstituted-3-methyl pyrazolo [4, 3-e]-pyrido [1, 2-a] pyrimidines *via* Michael addition and cycloelimination reactions

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A new series of novel 1, 4-disubstituted-3-methyl pyrazolo [4,3-e]-pyrido [1,2-a] pyrimidines have been synthesized from a common intermediate, in good yields. These compounds have been screened for their antibacterial and antifungal activity against different pathogenic strains of bacteria and fungi. The minimum inhibitory concentration (MBC) and minimum fungicidal concentration (MFC) have been determined for the test compounds as well as for reference standards. Compounds **3d**, **3e**, **3f**, **3h** have shown good antibacterial activity whereas compounds **3a**, **3b**, **3c**, **3g** have displayed better antifungal activity.

Keywords: 1, 4-Disubstitutedaroyl/aroyloxy methyl, Schiff base, pyrazolo, pyrido [1,2-a]pyrimidine, antimicrobial, antibacterial activity, antifungal activity

Michael addition of nucleophiles to electron deficient alkenes is one of the most powerful and widely used synthetic tools for the formation of carbon-carbon and carbon-hetero bonds in organic chemistry[1-5]. Hetero Michael additionsare the most exploited organic reactions and are the mainstay of efficient synthetic tools for the construction of druggable heterocyclic scaffolds and natural products[6-8]. Construction of molecular architecture by two or more bond formation in one-step operation via Michael reaction has been one of the current interest in synthetic organic chemistry[9,10]. Azole containing compounds exhibit both antibacterial and antifungal activities and some of them are in clinical practice as antimicrobial agents. Because of the extensive use of these azole drugs, some microbial strains have developed resistance to these drugs. The increasing number of azole drug resistant strains has initiated research to develop new antimicrobial compounds. Some pyrazole derivatives are extensively studied and used as antimicrobial agents[11-17]. Pyrazoles are an important class of heterocyclic compound and many pyrazole derivatives are reported to have broad spectrum of biological activities like anti-inflammatory[18,19] antifungal[20], herbicidal[21,22], antitumor, cytotoxic[23], and antiviral[24,25] activities. Pyrozole derivatives also act as antiangiogenic agents[26], A3 adenosine receptor antagonists[27]. The heterocyclic fusion of pyrimidine and pyridine rings resulted in formation of pyridopyrimidines, the structural analogs of biogenic quinazolines and pteridines.

Pyridopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. Also, due to the presence of pyridopyrimidine moiety in some important drugs, interest in the construction of such molecules has been aroused. In the last few years, an enormous number of papers and reviews have been reported dealing with the chemistry and application of this class of compounds[28-31]. Pyrido [1, 2-a] pyrimidine isomers are found to be biologically active in a wide range such as antimalarial agents[32], psychotropic agents[33,34], anti allergic agents[35], the human leukocyte elastase inhibitor[36], anti-ulcer agent[37], CNS stimulants[38], urease inhibitor[39] and aggregation of human platelets inhibitors[40]. The high therapeutic properties of the compounds incorporating nitrogen heterocyclic have encouraged the medicinal chemists to synthesize large number of novel therapeutic agents.

In light of the above literature and abundance on bio-potentials of pyrazolo and pyridopyrimidine analogues, we designed the synthesis of titled compounds and were confident that these frame work would provide the important structural motifs for the discovery of new antimicrobial agents. In

continuation of our research an efficient synthesis of biologically active small molecules[41], we developed an efficient synthesis of 1, 4-disubstituted-3-methyl pyrazolo [4, 3-e]-pyrido [1, 2-a] pyrimidine derivatives and demonstrated their antimicrobial activity. The structure of these compounds was established by the IR, ^{1}HNMR spectral data and elemental analysis (Scheme I).

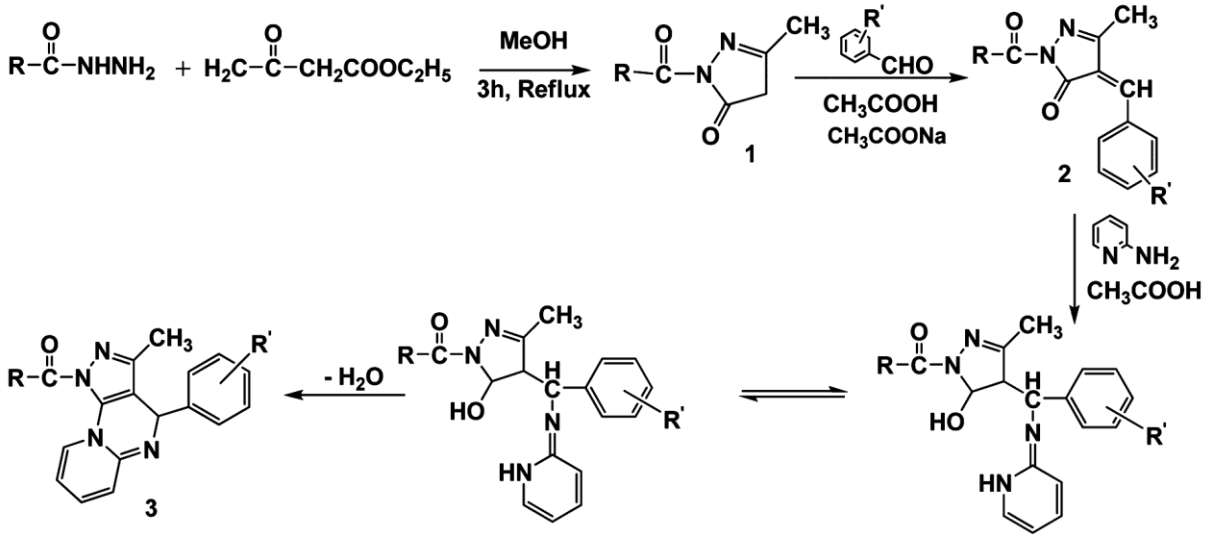
A plausible mechanism for the formation of titled 1, 4-disubstituted-3-methyl pyrazolo [4, 3-e]-pyrido [1, 2-a] pyrimidine is given in (Scheme II).

The required starting material 1-aroyl/aroyloxy-4-arylideno-3-methyl-pyrazolin-5-ones was prepared by the following known methods[42,17]. A mixture of 1-aroyl/aroyloxymethyl-3-methyl-pyrazolin-5-one, fused sodium acetate and an araldehyde in glacial acetic acid was refluxed for 2-3h. The reaction mixture was poured in to cold water, filtered, dried and recrystallised from methanol to furnish the corresponding pyrazolin derivatives.

Fungicidal Activity

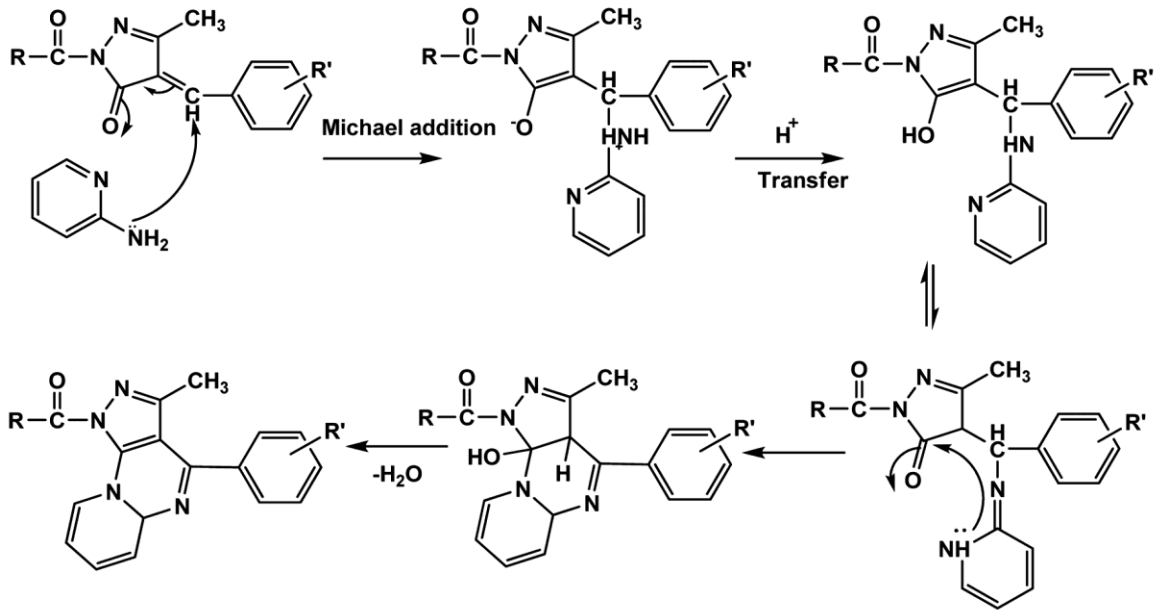
National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. NCCLS document M27-A. Wayne, Pa: National Committee for Clinical Laboratory Standards; 1997

The synthesized pure compounds were screened for their antifungal activities adopting standard protocols. The antifungal activity, of prepared final pure compounds was performed against *Pyricularia oryzae* (ATCC 15024) *Pseudoperonospora cubensis (ATCC 74149)*, *Sphaerotheca fuliginea (ATCC 74387*) and *Phytophthora infestans (ATCC 96155)* using Nystatin. Minimum inhibitory concentration (MIC) was determined and reported in μg/mL as positive and DMSO as negative control. Antifungal activity was carried out through broth diffusion method[43,] All fungal cultures were routinely maintained on Sabouraud dextrose agar (SDA) and incubated at 28°C.



	R	R'
A	4-ClC_6H_4	4-Cl
B	2,4-$Cl_2C_6H_3$	4-OCH_3
C	2-OHC_6H_4	4-OCH_3
D	4-$NO_2C_6H_4$	3,4-$(OCH_3)_2$
E	4-$NO_2C_6H_4$	4-Cl
F	$C_6H_5OCH_2$	4-OCH_3
G	$C_6H_5OCH_2$	3,4-$(OCH_3)_2$
H	4-$ClC_6H_4OCH_2$	4-Cl

Scheme I



Scheme II

The summarized data are presented in Table I, only for those compounds which were found active against any of these strains of fungi. It is evident from Table I that compounds **3a**, **3b**, **3c** and **3g** showed antifungal activity against used strains of fungi ranging from 9 to 20 μg/mL concentrations. The compound **3g**was the only active compounds against *P. Cubensis* and *S. fuliginea* in 20 and 14 ug/mL concentration respectively. Compound**3c** was found active against *P. oryzae* at 15 μg/mL concentration.

Antibacterial Activity

The newly prepared compounds were screened[44] for their antibacterial activity against *Staphylococcus aureus ,(ATCC 25923)*; *Escherichia coli* (ATCC 64) and *Klebsiella pneumonia (ATCC 424)* using ciprofloxacin as positive and DMSO as negative Control. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined and the activity was reported in μg/mL. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and controls was inoculated with approximately 5×10^5 c.f.u/mL of activity dividing bacteria cells. The cultures were incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. The antibacterial results in Table II are summarized only for those compounds which were found active against any strain of bacteria. It is evident from Table II that compounds

Table I — Antifungal activity of compounds**3a**, **3b**, **3c** and **3g**

Compd	Fungal species and MIC (g/mL)			
	P. oryzae	*P. cubensis*	*S. fuliginea*	*P. infestans*
3a	12	9	9	11
3b	11	11	9	10
3c	15	n.a.	n.a.	n.a.
3g	n.a.	20	14	n.a.
Nystatin	18	20	18	18
DMSO	n.a.	n.a.	n.a.	n.a.

MIC (g/mL), minimum inhibitory concentration i.e. the lowest concentration of the compounds to inhibit the growth of fungi.
n.a. - no activity detected.

Table II — Antibacterial activity of compoundsposeses promising biological activity

Compd	Gram-positive bacteria		Gram-negative bacteria			
	S. aureus		*E. coli*		*K. pneumonia*	
	MIC	MBC	MIC	MBC	MIC	MBC
3d	40	100	n.a.	n.a.	n.a.	n.a.
3e	30	100	n.a.	n.a.	n.a.	n.a.
3f	n.a.	n.a.	25	50		n.a.
3h	n.a.	n.a.	n.a.	n.a.	30	50
Ciprofloxacin	6.5	12.5	6.25	25	6.25	10.50
DMSO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

MIC (g/mL), minimum inhibitory concentration i.e. the lowest concentration of the compounds to inhibit the growth of bacteria completely.
MBC (g/mL) minimum bactericidal concentration i.e. the lowest concentration of the compound for killing the bacteria completely, n.a. – no activity detected.

3d and **3e** showed moderate activity against gram-positive bacteria *S. aureus* whereas **3f** was active against gram-negative *E. coli* ranging at 25 µg/mL and compound **3h** was found active against *K. pneumoniae* ranging from 30-50 µg/mL concentration (Table II).

The antifungal screening data of the tested compounds revealed that most of them showed weak to moderate activity. Although all of them have polar substituents like, Cl, 2,4- Cl_2 or -OH in their structures. The compound **no3g** having three - OCH_3 and one methoxypheyl groups showed some promising antifungal on *P. cubensis*. The antibacterial activity data reflects that none of the compounds of this series possess promising activity except compound **no 3f** which have notable activity on *E. coli*. Thus none of the compounds of this series possess antifungal and antibacterial activity comparable to commercial compounds tested under similar conditions.

Experimental Section

Melting points were recorded in Richerf-Thermover instrument and are uncorrected. The IR spectra were recorded on Parkin- Elmer- RXI spectrometer in KBr. ^1H and ^{13}C NMR spectra were recorded on Bruker 300 and BrukerAvance II 400 spectrometer using tetra methylsilane (TMS) as an internal standards and DMSO-d_6/$CDCl_3$ as solvent. The micro analytical data werecollected on Elemental Vario EL III element analyzer. All chemicals used were purchased from Merck and Fluka Chemicals. The homogeneity of compounds was checked by thin layer chromatography (TLC) on glass plates coated with silica gel G_{254} (Merck, Mumbai, India) using chloroform-methanol (3:1) mixture as mobile phase.

General procedure for synthesis of l-4-disubstituted- 3-methyl pyrazolo[4, 3-e]-pyrido[1, 2-a]pyrimidines

A mixture of 1-aroyl/aroyloxymethyl-4-arylideno-3-methyl pyrazolin-5-one (0.01 mol), 2-amino pyrimidine (0.01mol) in glacial acetic acid (10 mL) was refluxed for 8h. The resulting solution was cooled and poured in to water. The solid product obtained was filtered and washed with water, dried and purified by recrystallization from ethanol to get crystalline solid products **3a-h.**

3a: Colourless solid Yield 65% m.p. 205°C; IR: 3050(C-H arom), 1710 (C=O), 1275 (C=C of benzene ring)cm^{-1}; ^1H NMR (DMSO-d6): δ7.33-8.59 (m, 8 CH arom. proton), 6.12-6.20 (m 4H pyridine ring proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 15.2, 111.3,113.1,120.5,124.9, 129.6, 130.7, 134.5, 138.5, 141.9, 143.3, 146.2, 148.3, 151.6, 155.2, 156.0, 158.1, 160.8, 163.0, 163.7, 168.

Molecular formula $C_{23}H_{16}N_4OCl_2$, (m/z) (M^+): 434.880

3b: Colourless solid Yield 69% m.p. 201°C; IR: 3060 (C-H arom), 1725 (C=O), 1225 (C=C of benzene ring)cm^{-1}; ^1H NMR (DMSO-d6): δ 7.10-7.69 (m, 8 CH arom. proton),6.36-6.41 (m 4H pyridine ring proton),3.50 (s, 3H OCH3 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 55.9, 92.0,110.3,113.8,120.2,124.0, 129.2, 131.7, 133.5, 137.5, 141.5, 143.8, 145.2, 148.6, 150.6, 154.2, 158.0, 158.9, 160.8, 163.2, 164.7, 168.$C_{24}H_{18}N_4O_2Cl_2$, (m/z) (M^+): 465.332

3c: Colourless solid Yield 65% m.p. 216°C; IR: 3045 (C-H arom), 1715 (C=O), 1210 (C=C of benzenering)cm^{-1}; ^1H NMR (DMSO-d6): δ 7.00-7.94 (m, 8 CH arom. proton),6.26-6.38 (m 4H pyridine ring proton), 5.0 (s 1H hydroxyl proton), 3.50 (s, 3H OCH3 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 55.9, 92.0,114.3,115.8,121.2,124.9, 126.4, 129.2, 131.7, 133.6, 137.7, 141.1, 143.3, 145.5, 147.6, 150.8, 154.4, 158.4, 158.9, 160.8, 163.2, 164.7, 168.0 $C_{24}H_{20}N_4O_3$, (m/z) (M^+): 412.34

3d: Colourless solid Yield 53% m.p. 192°C; IR: 3035 (C-H arom), 1705 (C=O), 1205 (C=C of benzene ring)cm^{-1}; ^1H NMR (DMSO-d6): δ 7.35-8.34 (m, 7 CH arom. proton),6.0-6.17 (m 4H pyridine ring proton), 3.72 (s, 6H OCH3 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 56.9, 56.9, 92.2,114.4,115.5,121.1,124.4, 126.6, 129.9, 131.1, 133.3, 137.7, 141.9, 143.6, 145.7, 147.3, 151.8, 154.6, 158.0, 158.9, 160.8, 163.2, 164.7, 168.2 $C_{25}H_{21}N_5O_5$, (m/z) (M^+): 470.900

3e: Colourless solid Yield 70% m.p. 225°C; IR: 3020 (C-H arom), 1710 (C=O), 1210 (C=C of benzene ring)cm^{-1}; ^1H NMR (DMSO-d6): δ 7.30-8.38 (m, 8 CH arom. proton),6.10-6.21 (m 4H pyridine ring proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 92.2,112.0,115.3,121.0,124.2, 126.0, 129.2, 131.4, 133.5, 137.8, 141.6, 143.7, 145.8, 147.9, 151.4, 154.6, 158.2, 159.0, 160.8, 163.2, 164.5, 168.1 $C_{23}H_{17}N_3O_5Cl$, (m/z) (M^+): 445.52

3f: Colourless solid Yield 69% m.p. 190°C; IR: 3025 (C-H arom), 1690 (C=O), 1220 (C=C of benzene ring)cm^{-1}; ^1H NMR (DMSO-d6): δ 6.80-7.58

(m, 9 CH arom. proton),6.15-6.26 (m 4H pyridine ring proton), 4.79 (s 2H OCH_2 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 55.9, 74.6, 83.3, 92.2, 109.5,112.0,115.3, 120. 8, 127.0, 128.5, 129.2, 131.4, 133.5, 135.4, 136.6, 137.8, 141.6, 143.7, 145.8, 147.9, 151.4, 154.6, 158.2, $C_{25}H_{22}N_4O_3$, (m/z) (M^+): 425.645

3g: Colourless solid Yield 68% m.p. 165 °C; IR: 3025 (C-H arom), 1690 (C=O), 1220 (C=C of benzene ring)cm-^{1}; ^{1}H NMR (DMSO-d6): δ 6.80-7.58 (m, 9 CH arom. proton),6.18-6.25 (m 4H pyridine ring proton), 4.79 (s 2H OCH_2 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 55.9, 74.6, 83.3, 92.2, 109.5,112.0,115.3, 120. 8, 127.0, 128.5, 129.2, 131.4, 133.5, 135.4, 136.6, 137.8, 141.6, 143.7, 145.8, 147.9, 151.4, 154.6, 158.2, $C_{27}H_{26}N_4O_4$, (m/z) (M^+): 470.22

3h: Colourless solid Yield 60% m.p. 208°C; IR: 3025 (C-H arom), 1695 (C=O), 1255 (C=C of benzene ring)cm-^{1}; ^{1}H NMR (DMSO-d6): δ 6.69-7.19 (m, 8 CH arom. proton), 6.15-6.20 (m 4H pyridine ring proton), 4.79 (s 2H OCH_2 proton), 3.73 (s, 6H OCH_3 proton), 2.75 (s, *J* =4Hz ,3H, CH_3);^{13}C NMR (δ ppm) : δ 11.6, 56.2, 56.2, 74.6, 83.3, 92.2, 109.4,114.0,115.2, 122.8, 127.0,129.2, 132.4, 134.5, 136.4, 137.8, 138.9, 141.6, 143.7, 145.8, 146.2 147.9, 151.4, 154.6, 157.2, $C_{25}H_{19}N_4O_2Cl_2$, (m/z) (M^+): 478.122

Conclusion

In the present investigation, a series of new heterocycles have been synthesized and screened for their antifungal and antibacterial activity. The activity results reveal that the synthesized compounds possess moderate to good activity profiles. The insights gained in this study will be useful for development of newer anti-infective agents.

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