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Thermal solvent-free synthesis of novel pyrazolyl chalcones and pyrazolines as potential antimicrobial agents

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ABSTRACT

A novel approach was adopted for the synthesis of series of new pyrazolyl chalcones (**3a**–**c**) by the reaction of 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (**1**) with different 5-acetylbarbituric acid derivatives (**2a**–**c**) under thermal solvent-free condition. The chalcones were then converted to the corresponding pyrazolines (**4a**–**c**) under the same condition in excellent yields. All the synthesized compounds were characterized using elemental analysis and spectral data (IR, ^{1}H NMR, and mass spectrometry). The synthesized compounds were tested for their antimicrobial activity by disk diffusion assay with slight modifications against Gram-positive, Gram-negative strains of bacteria as well as fungal strains. The investigation of antimicrobial screening revealed that compounds (**3a**–**4c**) showed good antibacterial and antifungal activities, respectively. Among the screened compounds, **3b** showed more potent inhibitory activity (MIC = 12.5 μg/ml) nearly to that of standard antibiotics ciprofloxacin, griseofulvin and fluconazole.

Development of new antimicrobial agents with novel structure and mode of action remains the primary goal of scientists for the solution of increasing bacterial resistance gained by microorganism to classical antimicrobial agents.[1] Chalcones and their derivatives are an attractive molecular scaffold for the search of new biologically active molecules. Studies reveal that incorporation of pyrazole moiety into various heterocyclic ring systems gives worthwhile molecules from the biological point of view.[2–4] Several pharmaceutical drugs including celecoxib[5] and rimonabant[6] utilize the pyrazole as their core molecular entity.[7,8] Many pyrazole derivatives are reported to have a broad spectrum of biological activities, such as anti-inflammatory,[9] antifungal,[10] herbicidal,[11] antiviral,[12] A3 adenosine receptor antagonists,[13] etc. On the other hand chalcones or 1,3-diaryl/heteroaryl-2-propene-1-ones, have been recently subject of great interest due to their interesting pharmacological activities including antioxidant,[14] antibacterial,[15] antileishmanial,[16] anticancer,[17] antiangiogenic,[18] anti-infective, anti-inflammatory,[19] nitric oxide inhibition,[20] antifungal,[21] tyrosinase inhibition,[22] etc.

Among the various derivatives of chalcones, synthesis of pyrazolines has engrossed substantial attention from organic[23,24] and medicinal chemists because of their pronounced biological activities such as tranquillizing, muscle relaxant, psychoanaleptic, antidepressant, antimicrobial, etc.[25–32] Keeping in view the potential biological activities of pyrazoles, chalcones and pyrazolines, it was perceived that the synergistic effect of heterocyclic moieties in single nucleus might result in the formation of some worthwhile molecules from the biological point of view.

One of the challenges facing chemists this century is to develop new transformations that are not only efficient, selective, and high yielding but also environmentally benign.[33] During the last decade, the topic of 'green chemistry' has received increasing attention. 'Green chemistry' aims the total elimination (or at least the minimization) of waste, and the implementation of sustainable processes. The utilization of non-toxic chemicals, renewable materials and solvent-free conditions are the key issues of green synthetic strategy.[34] Thermal solvent-free reactions are gaining importance in this context as it meets the requirement of greenness.

To the best of our knowledge no reports have so far made in the synthesis of pyrazolyl chalcones and pyrazolines under thermal solvent-free 'green' condition. Thus, in accordance with the increasing interest in the medicinal chemistry community in technologies and concepts that facilitate a more rapid environmentally benign synthesis and consequently, the screening of novel chemical substances herein we report the novel synthesis of biologically important pyrazolyl chalcones and pyrazolines under thermal solvent-free condition in the absence of any acidic or basic catalyst. Furthermore, the newly synthesized compounds were screened for their in vitro antimicrobial activities against Gram-positive bacteria, Gram-negative bacteria and fungi.

Chalcones are usually synthesized by the Claisen–Schmidt reaction in basic/acidic medium in polar solvents and involves

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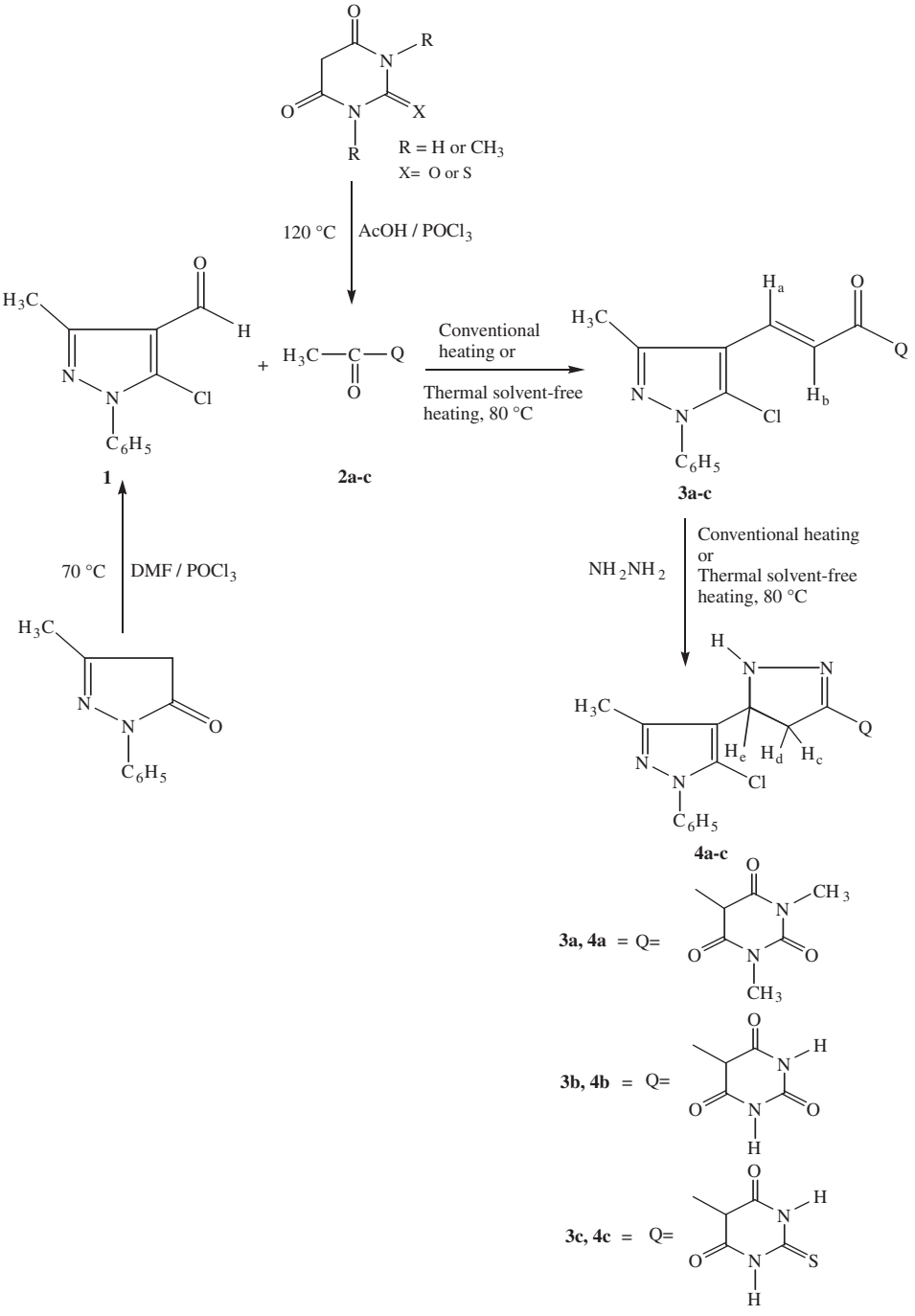
cumbersome purification process as the reaction often lead to a complex mixture. Many of these reported synthetic procedures require harsh reaction conditions, along with poor yields and low selectivity.[35] Due to exceptional reactivity of formyl group in 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde **1** as well as the versatile biological activities of barbituric acid derivatives,[36] heteroaryl chalcones **3a–c** were synthesized by the reaction of **1** with 5-acetyl-1,3-dimethylbarbituric acid (**2a**), 5-acetylbarbituric acid (**2b**) and 5-acetylthiobarbituric acid (**2c**) in a variety of solvents and in solvent-free condition (Scheme 1)[43]. The chalcones **3a–c** synthesized using ethanol/methanol/acetic acid as solvents in conventional method under the influence of basic catalyst, took longer period for completion of reaction (8–20 h) with lower yields (62–78%) (Table 1). In order to achieve the requirement of greenness, we then carried out reactions under thermal solvent-free condition by simply heating the starting materials at 80 °C. To our pleasant surprise, reactions were completed in shorter time (10–20 min) with substantial increase in yield of products (82–87%) even in the absence of any acidic or basic catalyst (Table 1). Thus, the present work also describes the superiority of green synthetic method over the conventional toxic solvent heating procedures. The structures of the compounds isolated were characterized by elemental and spectral analysis (IR, ^{1}H NMR and Mass spectrometry).

The infrared (IR) spectrum of **3a** showed the carbonyl absorption band of barbituric moiety at 1715 cm^{-1}. The carbonyl group



Scheme 1. Synthetic route of pyrazolyl chalcones (**3a–c**) and pyrazolyl pyrazolines (**4a–c**).

Table 1
Synthesis of compounds **3a**–**4c** under various reaction conditions

Entry	Product	Conventional heating methods						Thermal solvent-free method	
		Ethanol/pyridine, reflux		Methanol/piperidine, reflux		Acetic acid/sodium acetate, reflux			
		Time[a]	Yield[b](%)	Time[a]	Yield[b] (%)	Time[a]	Yield[b] (%)	Time[a]	Yield[b] (%)
1	**3a**	12 h	72	11.5	70	16 h	68	10 min	87
2	**3b**	14 h	70	12 h	70	20 h	64	20 min	82
3	**3c**	8 h	78	8 h	73	12 h	62	20 min	86
4	**4a**	45 min	68	25 min	64	1 h	72	5 min	89
5	**4b**	45 min	71	40 min	69	45 min	70	10 min	91
6	**4c**	35 min	66	35 min	68	45 min	76	10 min	90

[a] Reaction progress monitored by TLC.
[b] All yields refer to recrystallized products.

of propenone moiety appeared as strong and sharp absorption band at 1664 cm^{-1}. Another sharp and strongly absorbed band at 1618 cm^{-1} was assigned to carbon–carbon double bond of α,β-unsaturated system. The ^1H NMR spectrum showed *trans* olefinic protons H_a and H_b as *ortho* coupled doublets at δ 8.00 (J = 16.2 Hz) and δ 8.55 (J = 15.9 Hz), respectively. The value of spin–spin coupling constant J_{ab} in the range 15–16 Hz is indicative of the *E*-configuration of chalcone. The aromatic protons of the *N*-phenyl-pyrazole-moiety were present in the form of multiplet at δ 7.42–7.58. The two N–CH_3 group protons of barbituric acid moiety were discernible as two sharp singlets at δ 3.37 and 3.40 whereas protons of CH_3 group of pyrazole unit appeared as another sharp singlet at δ 2.59. Further confirmation of the structure was given by mass spectra, which showed M^+ at 400.12 as base peak. The spectral data of other compounds followed similar pattern.

With the objective of synthesizing pyrazolines containing pyrazole moieties heterochalcones, (**3a**–**c**) were treated with hydrazine hydrate in ethanol/methanol/acetic acid under basic medium in conventional heating method (Scheme 1)[44]. The reactions as visualized afforded the products (**4a**–**c**) in considerable yields (64–76%) within 25 min–1 h (Table 1). The same reactions carried out under solvent-free condition afforded the products (**4a**–**c**) with increase in yields (89–91%) along with substantial reduction in reaction time (5–10 min) (Table 1). All the reactions carried out in conventional solvents were found to be incomplete in the absence of the basic/acidic catalysts, however thermal solvent-free reactions proceeded smoothly in the absence of catalysts. Thus, our methodology added a new efficient procedure for the generation of novel pyrazolyl chalcones and pyrazolines in excellent yields, to the existing conventional solvent heating techniques.

The IR spectrum of **4a** showed a broad absorption band at 3200 cm^{-1} indicating the NH group of pyrazoline moiety. The carbonyl absorption band of barbituric acid unit was present at 1709 cm^{-1}. The absorbtion bands at 1633,1363 cm^{-1} were assigned to C=N, C–N groups of pyrazoline moiety, respectively. In ^1H NMR spectrum of **4a**, the presence of the pyrazoline unit was manifested by two doublets of doublets at δ 3.58 (H_d), 4.05 (H_c) and a triplet at 4.80 (H_e). The NH proton of pyrazoline unit appeared as broad singlet at δ 6.95. The five aromatic protons of *N*-phenyl-pyrazole-moiety were discernible as multiplets in the range of δ 7.48–7.52. The six N–CH_3 protons were present as sharp singlet at δ 3.34 whereas protons of CH_3 group of pyrazole unit appeared at δ 2.24 as another sharp singlet. Further evidence of the structure was given by mass spectrometry, which has given M^+ ion peak at 414.15 as base peak. The spectral data of other pyrazolines as obtained are explained in references and notes.

Melting points were taken in Reichert Thermover instrument and are uncorrected. The IR spectra were recorded on Perkin Elmer RXI spectrometer in KBr, ^1H NMR on Bruker DRX-300 and Bruker Avance II-400 spectrometer using tetramethyl silane (TMS) as the internal standard and DMSO-d_6/$CDCl_3$ as solvent. Mass spectra were recorded on JEOL-Accu TOF JMS-T100LC DART-MS spectrometer, Micromass Quattro II triple quadrupol mass spectrometer and Jeol-SX-102 (FAB) spectrometer. Elemental analyzes (C, H, N) were conducted using Carlo Erba analyzer model 1108. The purity of all compounds was checked by TLC on glass plates (20 × 5 cm) coated with silica gel (E-Merck G_{254}, 0.5 mm thickness). The plates were run in chloroform–methanol (4:1) mixture and were visualized by iodine vapors. 3-Methyl-1-phenylpyrazole-5-one, barbituric acid, thiobarbituric acid and 1,3-dimethylbarbituric acid were purchased from Sigma–Aldrich chemicals Pvt. Ltd. Other chemicals were of commercial grade and used without further purification. 5-Chloro-3-methyl-1-phenylpyrazole-4- carboxaldehyde,[37] 5-acetylbarbituric acid, 5-acetylthiobarbituric acid and 5-acetyl-1,3-dimethylbarbituric acid[38] were synthesized by using DMF/$POCl_3$ and AcOH/$POCl_3$ according to the reported methods.

Antibacterial studies: The newly synthesized compounds were screened for their antibacterial activity against *Streptococcus pyogenes* (clinical isolate), Methicillin resistant *Staphylococcus aureus* (MRSA +Ve), *Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneumoniae* (clinical isolate) and *Escherichia coli* (ATCC-25922) bacterial strains by disk diffusion method.[39,40] A standard inoculums (1–2 × 10^7 c.f.u/ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Ciprofloxacin (30 μg) was used as positive control while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 × 10^5 c.f.u/ml of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bactericidal concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 3.

The investigation of antibacterial screening data revealed that all the tested compounds **3a**–**4c** showed moderate to good bacterial inhibition. All the compounds showed good inhibition

Table 2
Antibacterial activity of compounds **3a–4c**

Compounds	Diameter of zone of inhibition (mm)				
	Gram-positive bacteria		Gram-negative bacteria		
	S. pyogenes	*MRSA*[a]	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
3a	20.1 ± 0.4	17.3 ± 0.4	25.2 ± 0.3	16.1 ± 0.4	22.2 ± 0.4
3b	22.2 ± 0.2	20.2 ± 0.2	27.2 ± 0.4	17.1 ± 0.2	24.8 ± 0.2
3c	18.4 ± 0.4	17.1 ± 0.3	22.1 ± 0.3	15.4 ± 0.3	20.7 ± 0.3
4a	17.1 ± 0.2	16.5 ± 0.2	21.8 ± 0.2	15.7 ± 0.4	19.6 ± 0.2
4b	16.8 ± 0.1	15.3 ± 0.6	20.1 ± 0.5	14.7 ± 0.4	19.2 ± 0.4
4c	15.2 ± 0.1	14.2 ± 0.2	18.2 ± 0.4	14.4 ± 0.3	19.1 ± 0.3
Standard	24.0 ± 0.3	25.0 ± 0.4	32.0 ± 0.3	18.0 ± 0.2	26.0 ± 0.4
DMSO	—	—	—	—	—

Positive control (standard): ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).
[a] Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

Table 3
MIC and MBC results of **3a–4c** and positive control ciprofloxacin

Compounds	Gram-positive bacteria				Gram-negative bacteria					
	S. pyogenes		*MRSA*		*P. aeruginosa*		*K. pneumoniae*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3a	25	50	25	50	25	100	50	100	25	50
3b	12.5	25	12.5	50	12.5	25	12.5	25	12.5	50
3c	25	100	25	50	25	100	25	50	50	100
4a	25	50	50	100	50	100	50	100	25	100
4b	50	100	100	>100	50	100	50	>100	50	>100
4c	50	100	100	>100	50	100	50	>100	50	100
Standard	12.5	50	12.5	50	12.5	25	12.5	50	12.5	50

MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bactericidal concentration, that is, the lowest concentration of the compound for killing the bacteria completely.

against *S. pyogenes*, Methicillin resistant *S. aureus* (MRSA +Ve), *P. aeruginosa*, *K. pneumoniae* and *E. coli* species. Compound **3b**, showed more potent antibacterial activity (MIC = 12.5 μg/ml) nearly equivalent to that of ciprofloxacin against all bacterial strains tested. The MBC of compounds was found to be two, three or four folds higher than the corresponding MIC results. A comparative study also revealed that pyrazolyl chalcones **3a–c**, are more potent antimicrobial agents than pyrazolines **4a–c**. The potential bactericidal effects of chalcones can be related to the ability of the α,β-unsaturated ketone system to undergo a conjugated addition to a nucleophilic group like a thiol group in an essential protein.

Antifungal studies: Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes* and *Penicillium marneffei* were recultured in DMSO by agar diffusion method.[41,42] Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty milliliter of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The antifungal activity of each compound was compared with griseofulvin and fluconazole as standard drugs. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4 - 6 \times 10^4$ c.f.u/ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 5.

Table 4
Antifungal activity of compounds (**3a–4c**): Positive control (griseofulvin, fluconazole) and negative control (DMSO) measured by the Halo Zone Test (unit, mm)

Compounds	Diameter of zone of inhibition (mm)			
	CA	AF	TM	PM
3a	21.3 ± 0.4	18.6 ± 0.4	17.2 ± 0.2	14.8 ± 0.4
3b	25.5 ± 0.5	21.3 ± 0.2	17.1 ± 0.3	14.1 ± 0.2
3c	20.4 ± 0.3	17.9 ± 0.3	17.1 ± 0.6	14.1 ± 0.3
4a	20.8 ± 0.4	17.4 ± 0.2	16.9 ± 0.4	14.2 ± 0.6
4b	20.4 ± 0.5	16.8 ± 0.3	16.0 ± 0.2	13.1 ± 1.2
4c	20.2 ± 0.2	16.2 ± 0.3	15.8 ± 0.2	13.1 ± 0.2
Griseofulvin	30.0 ± 0.2	27.0 ± 0.2	24.0 ± 0.3	20.0 ± 0.5
Fluconazole	20.0 ± 0.5	20.0 ± 0.5	19.0 ± 0.5	18.0 ± 0.5
DMSO	—	—	—	—

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*.

The antifungal screening data of the compounds revealed good to moderate activity. Compounds **3a**, **3b**, and **3c** were showed a good inhibitory activity against *C. albicans* and *A. fumigatus*. Compounds **3a–c** also showed moderate activity towards *T. mentagrophytes* and *P. marneffei* fungal strains. Compounds **4a**, **4b**, and **4c** were less active towards *C. albicans*, *A. fumigatus*, *T. mentagrophytes* and *P. marneffei* fungal strains. Among the screened compounds, **3b**

Table 5
MIC and MFC of compounds **3a–4c**, positive control griseofulvin, fluconazole

Compounds	CA		AF		TM		PM	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
3a	25	50	25	50	25	50	25	50
3b	12.5	25	12.5	25	12.5	25	12.5	25
3c	25	50	25	50	25	50	25	100
4a	25	100	25	100	25	100	50	>100
4b	50	100	50	100	50	100	50	100
4c	50	100	25	100	25	100	>100	>100
Gris	6.25	12.5	12.5	25	6.25	25	12.5	25
Flu.	6.25	12.5	6.5	12.5	6.25	12.5	6.5	12.5

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (μg/ml) = minimum fungicidal concentration, that is, the lowest concentration of the compound for killing the fungus completely.

showed more potent inhibition against all the fungal strains (MIC = 12.5 μg/ml). The MFC of compounds were found to be two or three or four folds higher than the corresponding MIC results. Thus, overall the data revealed that the compounds **3a–4c** have produced the marked enhancement in the potency of these analogs as antibacterial and antifungal agents. A comparative study with known chalcones and pyrazolines revealed that the newly synthesized compounds are equally potent as most of the reported compounds. However, the mode of action of these compounds and their effect on mammalian cell still needs to be evaluated.

The fact that readily synthesized starting materials, available reagents along with short reaction time, no additives and simple work-up and isolation of the products under green condition make the current method a feasible and attractive protocol for generation of series of pyrazole derivatives in good yields. Further, in vitro antibacterial and antifungal evaluation of compounds has proved them as potent antimicrobial agents. The importance of such kind of work lies in the possibility that the new compounds might be more effective against microbes for which a thorough study regarding the structure–activity relationship, toxicity and their biological effects would be helpful in designing more potent antimicrobial agents.

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43. General method for the preparation of pyrazolyl chalcones (**3a–c**) under conventional heating conditions.
To a well stirred solution of 5-acetyl-1,3-dimethylbarbituric acid (4.52 mmol)/5-acetylbarbituric acid (4.52 mmol)/5-acetylthiobarbituric acid (4.52 mmol) in ethanol (15 ml)/methanol (15 ml)/acetic acid (12 ml), containing pyridine (1 ml)/piperidine (0.5 ml)/sodium acetate (4 mmol), 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (4.52 mmol) was added in portion. The reaction mixture was then refluxed in a heating mantle for specified time (Table 1) and cooled at room temperature. The yellow solid, thus, obtained was filtered, washed with water, alcohol and dried to afford **3a–c**. Further purification was made by recrystallization from chloroform-methanol (4:1v/v) mixture.
*General method for the preparation of pyrazolyl chalcones (**3a–c**) under thermal heating conditions.*
A mixture of 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde (4.52 mmol) and 5-acetyl-1,3-dimethylbarbituric acid (4.52 mmol)/5-acetylbarbituric acid (4.52 mmol)/5-acetyl thiobarbituric acid (4.52 mmol) was mixed well using a mortar and pestle. The reaction mixture was then transferred into a 50 ml R.B flask and heated at 80 °C in a heating mantle for specified time (Table 1). The progress of reaction was monitored by TLC. On completion, reaction mixture was cooled and 25 ml of ice cold water added. The bright yellow solid, thus, obtained was filtered, washed with water, alcohol and dried to afford **3a–c**. Further purification was made by recrystallization from chloroform–methanol (4:1v/v) mixture.
*(2E)-3-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-1-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)-2-propen-1-one (**3a**)*
Yellow crystals; yield 87%, mp 263–266 °C. IR (ν_{max}, cm^{-1}, KBr): 1618 (C=C), 1664 (C=O), 1715 (C=O). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.59 (3H, s, CH_3), 3.37 (3H, s, N–CH_3), 3.40 (3H, s, N–CH_3), 7.42–7.58 (5H, m, Ar-H), 8.00 (1H, d, J = 16.2 Hz, H_a), 8.55 (1H, d, J = 15.9 Hz, H_b). ESI-MS m/z: 400.12 (M^+). Anal. Calcd for $C_{19}H_{17}N_4O_4Cl$: C, 56.93; H, 4.27; N, 13.97. Found: C, 56.76; H, 4.38; N, 13.91.

*(2E)-3-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-1-(2,4,6-pyrimidinetrione-5-yl)-2-propen-1-one (**3b**)*

White powder; Yield 82%, mp >300 °C. IR (ν_{max}, cm^{-1}, KBr): 1601 (C=C), 1657 (C=O), 1698 (C=O), 3193 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.62 (3H, s, CH_3), 7.47–7.85 (5H, m, Ar-H), 8.32 (1H, d, J = 15.8 Hz, H_a), 9.01 (1H, d, J = 15.8 Hz, H_b), 11.45 (1H, s, NH), 11.60 (1H, s, NH), 16.89 (1H, s, OH, D_2O exchangable). FAB-MS m/z(%): 372 (M^+, 75), 371 (55), 336 (10), 261 (20), 260 (10), 232 (40), 214 (15), 157 (100). Anal. Calcd for $C_{17}H_{13}N_4O_4Cl$: C, 54.77; H, 3.51; N, 15.02. Found: C, 54.86; H, 3.64; N, 15.04.

*(2E)-3-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-1-(2-mercapto-4,6-pyrimidinedione-5-yl)-2-propen-1-one (**3c**)*

Yellow powder; yield 86%, mp >300 °C. IR (ν_{max}, cm^{-1}, KBr): 1057 (C=S), 1608 (C=C), 1647 (C=O), 1746 (C=O), 3188 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.56 (3H, s, CH_3), 7.47–7.87 (5H, m, Ar-H), 7.87 (1H, d, J = 15.7 Hz, H_a), 9.04 (1H, d, J = 15.8 Hz, H_b), 12.19 (1H, s, NH), 12.73 (1H, s, NH). ESI-MS m/z: 388.6 (M^+), 361.6, 360.6, 310.6, 274.6, 202.6. Anal. Calcd for $C_{17}H_{13}N_4O_3SCl$: C, 52.51; H, 3.36; N, 14.40. Found: C, 52.57; H, 3.39; N, 14.32.

44. General method for the preparation (**4a**–**c**) under conventional heating conditions.

A mixture of **3a**–**c** (2.52 mmol) and hydrazine hydrate (2.52 mmol) in ethanol (10 ml)/pyridine (0.6 ml) or methanol (10 ml)/piperidine (0.4 ml) or acetic acid (8 ml)/sodium acetate (2 mmol) was refluxed in a heating mantle for specified time (Table 1). After completion of the reaction (checked by TLC), the reaction mixture was cooled and the solid precipitated out was filtered, washed with water, acetone and dried to afford **4a**–**c**. Further purification was made by recrystallization from chloroform–methanol (3:2) mixture.

*General method for the preparation of pyrazolines (**4a**–**c**) under thermal heating conditions*

The mixture of **3a**–**c** (2.49 mmol) and hydrazine hydrate (2.49 mmol) was mixed well using a mortar and pestle. The reaction mixture was then transferred in to a 50 ml R.B flask and heated at 80 °C over a heating mantle for specified time (Table 1). After completion of the reaction (checked by TLC), the reaction mixture was cooled and 20 ml of ice cold water was added. The white solid, thus, obtained was filtered, washed with water, acetone and dried to afford **4a**–**c**. Further purification was made by recrystallization from chloroform–methanol (3:2) mixture.

*5-(5-Chloro-3-methyl-1-phenylpyrazol-4-yl)-3-(1,3-dimethyl-2,4,6-pyrimidinetrione-5-yl)pyrazoline (**4a**)*

White crystals; yield 89%, mp >300 °C. IR (ν_{max}, cm^{-1}, KBr): 1363 (C–N), 1633 (C=N), 1709 (C=O), 3200 (NH). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.24 (3H, s, CH_3), 3.34 (6H, s, 2 N–CH_3), 3.58 (1H, dd, J = 18.6 Hz, 9.3 Hz, H_d), 4.05 (1H, dd, J = 18.9 Hz, 10.5 Hz, H_c), 4.80 (1H, t, J = 18.3 Hz H_e), 6.95 (1H, s, NH), 7.48–7.52 (5H, m, Ar-H). ESI-MS m/z: 414.15 (M^+), 413.15, 412.15, 277.15. Anal. Calcd for $C_{19}H_{19}N_6O_3Cl$: C, 55.01; H, 4.61; N, 20.25. Found: C, 55.13; H, 4.78; N, 20.13.

*5-(5-Chloro-3-methyl-1-phenylpyrazol-4-yl)-3-(2,4,6-pyrimidinetrione-5-yl) pyrazoline (**4b**)*

White powder; yield 91%, mp >300 °C. IR (ν_{max}, cm^{-1}, KBr): 1294 (C–N), 1616 (C=N), 1727 (C=O), 3169 (NH), 3215 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.32 (3H, s, CH_3), 3.74 (1H, dd, J = 18.9 Hz, 8.7 Hz, H_d), 4.07 (1H, dd, J = 19.0 Hz, 10.1 Hz, H_c), 4.93 (1H, t, J = 18.7 Hz, H_e), 7.33 (1H, s, NH), 7.41–7.66 (5H, m, Ar-H), 10.23 (1H, s, NH), 11.12 (1H, s, NH). 16.09 (1H, s, OH, D_2O exchangable). ESI-MS m/z: 386.6 (M^+), 376.6, 362.6, 361.6, 360.6, 358.6, 332.6, 318.6, 301.5, 274.6, 202.6. Anal. Calcd for $C_{17}H_{15}N_6O_3Cl$: C, 52.79; H, 3.90; N, 21.72. Found: C, 52.86; H, 3.98; N, 21.64.

*5-(5-Chloro-3-methyl-1-phenylpyrazol-4-yl)-3-(2-mercapto-4,6-pyrimidinedione-5-yl)pyrazoline (**4c**)*

White powder; Yield 90%, mp >300 °C. IR (ν_{max}, cm^{-1}, KBr): 1011 (C=S), 1313 (C–N), 1633 (C=N), 1688 (C=O), 3126 (NH), 3234 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.42 (3H, s, CH_3), 3.69 (1H, dd, J = 18.6 Hz, 8.5 Hz, H_d), 4.09 (1H, dd, J = 18.9 Hz, 10.1 Hz, H_c), 4.93 (1H, t, J = 18.7 Hz, H_e), 7.35 (1H, s, NH), 7.47–7.61 (5H, m, Ar-H), 11.51 (1H, s, NH), 11.92 (1H, s, NH). ESI-MS m/z: 402.6 (M^+), 388.6, 362.6, 361.6, 360.6, 358.6, 356.6, 332.6, 302.5, 301.5, 274.6, 202.6, 158.5. Anal. Calcd for $C_{17}H_{15}N_6O_2SCl$: C, 50.68; H, 3.74; N, 20.86. Found: C, 50.73; H, 3.85; N, 20.77.