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Synthesis and antimicrobial activities of some new pyrazole derivatives

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ABSTRACT

Chloro acetyl and Acetyl pyrazoline derivatives have been synthesised by the condensation of pyrazoline derivatives (obtained by the reaction of various chalcones with hydrazine hydrate) with chloroacetyl chloride and glacial acetic acid respectively. The structures of new compounds were established on the basis of elemental IR and H^1NMR data, the compounds were evaluated for their antibacterial activities.

Keywords: Antimicrobial activity, Chalcone, Pyrazole.

INTRODUCTION

Pyrazoline derivatives have displayed wide range of biological and pharmacological activities as analgesic, anti-inflammatory, antipyretic, antiparasitic, antimalarial, antifungal, antimicrobial and enzyme inhibitory agents[1]. Keeping in view the importance of these biological activities, it was considered of interest to synthesize some new acetyl and chloroacetyl derivatives of pyrazoles.

In the present work various chalcones(a) were prepared by condensing different combinations of aromatic aldehydes and ketones. These chalcones were cyclized in the presence of hydrazine hydrate to give pyrazoline derivatives(b) which is achieved by reacting chalcones with hydrazine hydrate by refluxing for 9-10hrs. This is followed by treatment with either chloroacetyl chloride or glacial acetic acid to obtain a set of chloroacetyl pyrazoline derivatives(c) and a set of acetyl pyrazoline derivatives (d).

The structures of the synthesised compounds are assigned on the basis of elemental analysis, IR and H^1NMR spectral data. The antimicrobial profile of the compounds synthesised has been studied against several microbes.

MATERIALS AND METHODS

The melting points of the compounds were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plate. The IR spectra (cm^{-1}) were recorded using KBr on a Fourier Transform IR spectrometer (model Shimadzu 8400s) in the range of 400-4000

^{1}H-NMR spectra were recorded in Brookfield 200 MHz-NMR spectrometer (Astrazeneca India Ltd) using $CDCl_3$ and chemical shifts(δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS).

General procedure for synthesis of chalcones[2].
A solution of 22g of sodium hydroxide in 200ml of water and 100g(122.5ml) of rectified spirit was kept in a bath of crushed ice, (0.43mol) of freshly distilled aromatic ketone was added with stirring, and then (0.43mol) of pure aromatic aldehyde was added dropwise with stirring, temperature of the mixture was maintained at about 25^0C and stirred vigorously until the mixture is so thick that stirring is no longer effective (2-3 hrs), reaction mixture was removed and kept in the refridgerator overnight. The product was filtered, washed with cold water until the washings are neutral to litmus. The crude chalcone, after drying in the air was recrystallised from rectified spirit.

IR Spectrum Showed the characteristic Absorption Peak at (cm^{-1}) 1658(C=O), 1323(CH=CH) Aromatic, 438(C=C).
^{1}H-NMR ($CDCl_3$):δ7.39-8.2(m, Ar- H), 7.64(d,COCH=CH),7.5(d,COCH=CH)

General procedure for synthesis of Pyrazoline derivatives [3]**.**
A mixture of chalcone (5mmoles) and hydrazine hydrate (5mmoles) was dissolved in absolute alcohol (25ml) and refluxed for 9-10 hrs. The reaction mixture was poured onto crushed ice and stirred, the solid thus obtained was filtered off and washed with water and taken for the next step immediately.

General procedure for synthesis of chloroacetyl pyrazoline derivatives[4].
Pyrazoline derivative (0.01mole) was dissolved in beaker containing 20ml of dry benzene placed on a mechanical stirrer. In another beaker containing 20ml of dry benzene, chloroacetyl chloride (0.01mole), was added and this solution was added dropwise with stirring to the other beaker containing pyrazoline derivative, stirring was continued vigorously until the mixture is so thick that stirring is no longer effective (2-3 hrs). Then removed and crushed ice was added and after few hrs the product was filtered off, washed with water and recrystallised using appropriate solvent.

IR Spectrum showed the characteristic absorption peak at (cm^{-1}):3037Aryl(C-H), 680(C=O), 1332(C-N), 1265(CH_2Cl), 682(R-Cl).

^{1}H-NMR ($CDCl_3$): δ3.8(s, 3H, OCH3), 4.5(s, CH_2Cl), 3.12(dd, CH_2 pyrazoline).

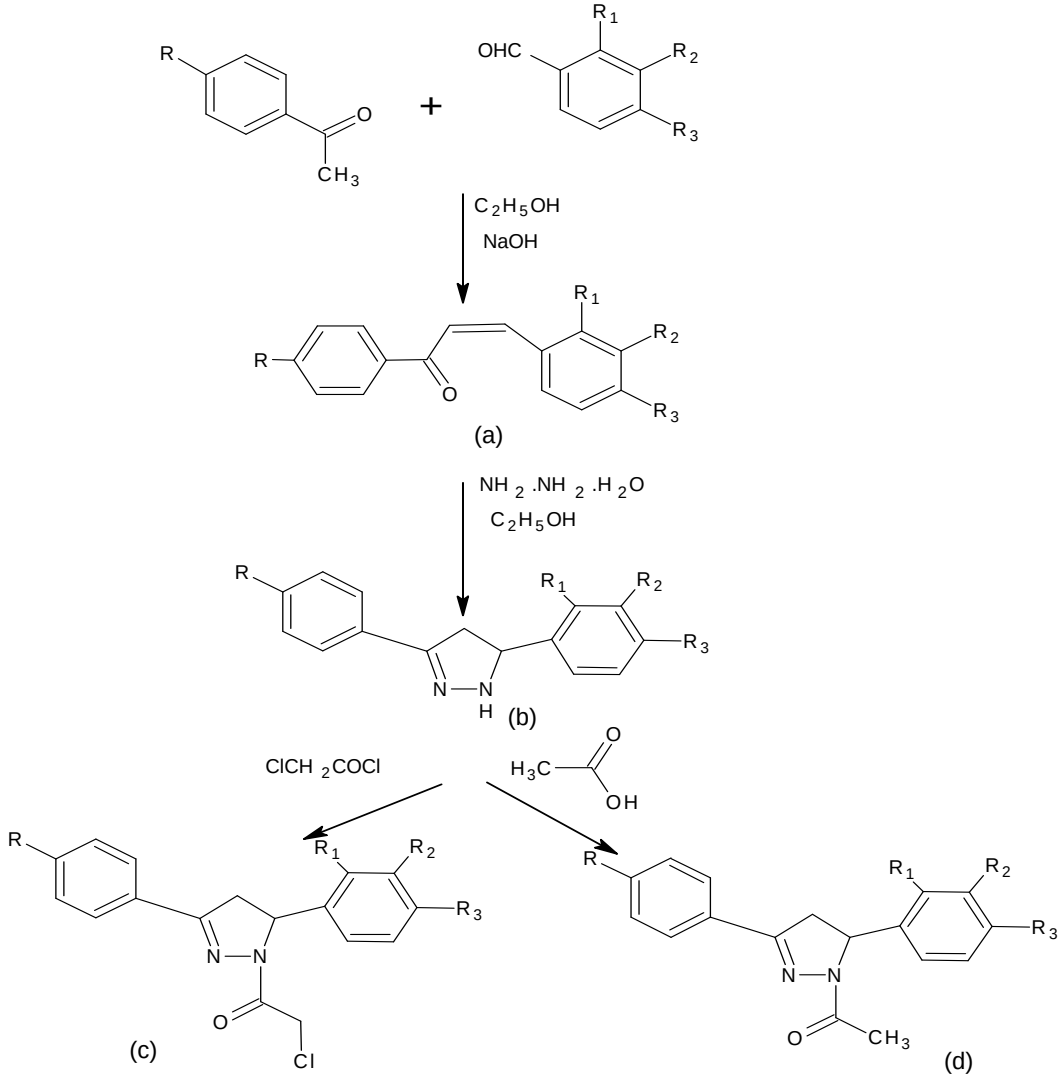
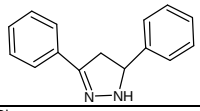
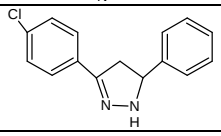
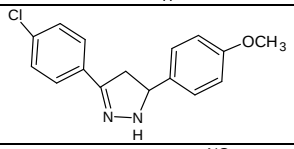
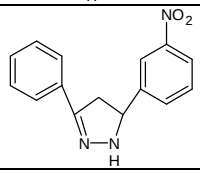
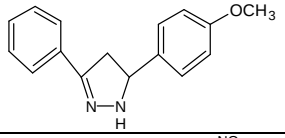
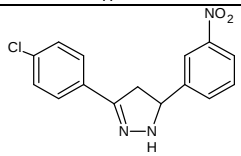
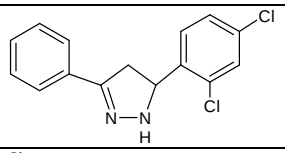
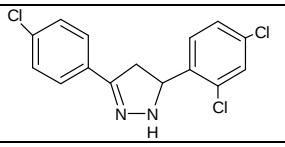


Table 1. List of the compounds synthesised

Compound Code	R	R_1	R_2	R_3
1A	H	H	H	H
2A	Cl	H	H	H
3A	Cl	OCH_3	H	H
4A	H	H	NO_2	H
5A	H	OCH_3	H	H
6A	Cl	H	NO_2	H
7A	Cl	H	H	H
8A	H	Cl	H	Cl
9A	Cl	Cl	H	Cl
10A	Cl	OCH_3	H	H

Table No.2 Physical Properties of the Pyrazoline derivatives

Sl. No.	Structure	Mol. Formula	IUPAC Name	Mol. Wt	TLC Solvent	% yield
1.		$C_{15}H_{14}N_2$	3,5-Diphenyl-4,5-dihydro-1*H*-pyrazole	293	Pet.Ether:DCM 2:1	70
2.		$C_{15}H_{13}ClN_2$	3-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole	256	"	72
3.		$C_{16}H_{15}ClN_2O$	3-(4-Chlorophenyl)-5-(4-methoxyphenyl) -4,5-dihydro-1*H*-pyrazole	286	"	75
4.		$C_{15}H_{13}N_3O_2$	5-(3-Nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	267	"	80
5.		$C_{16}H_{16}N_2O$	5-(4-Methoxyphenyl) -3-phenyl-4,5-dihydro-1*H*-pyrazole	252	"	80
6.		$C_{15}H_{12}ClN_3O_2$	3-(4-Chlorophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1*H*-pyrazole	301	"	75
7.		$C_{15}H_{12}Cl_2N_2$	5-(2,4-Dichlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	291	"	75
8.		$C_{15}H_{11}Cl_3N_2$	3-(4-Chlorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole	325	"	70

General procedure for synthesis of acetyl pyrazoline derivatives.

A mixture of Pyrazoline derivative (0.01moles) and glacial acetic acid (10ml) was heated under gentle reflux for 3hrs. The solution was poured in ice Cold water, the solid thus obtained was collected and crystallized using appropriate solvent.

IR Spectrum showed the characteristic absorption peak at (cm^{-1}):3065(Aryl C-H), 1664(C=O),1581(C=N),1334(CH_3bend) ^{1}H-NMR ($CDCl_3$): δ2.4(s, 3H, COCH3), 3.17(dd, CH_2 pyrazoline), 7.2-7.7(m, Ar-H).

Table No.3 Physical Properties of the Chloroacetylated Pyrazoline derivatives

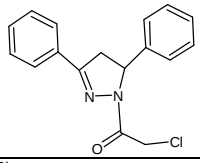
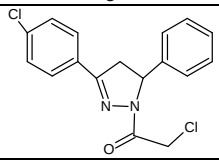
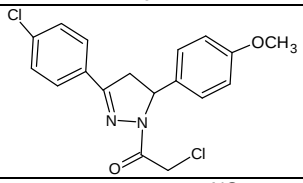
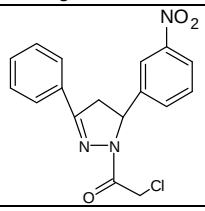
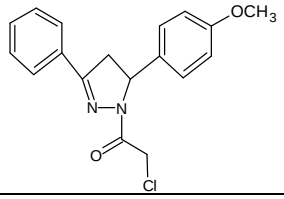
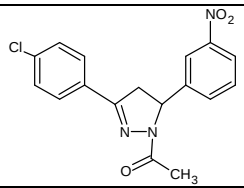
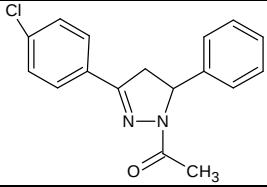
Compd Code.	Structure	Mol. Formula	IUPAC Name	Mol. Wt.	M.P	% Yield
1A		$C_{17}H_{15}ClN_2O$	1-Chloroacetyl)-3,5-diphenyl-4,5-dihydro-1*H*-pyrazole	338	115	65
2A		$C_{17}H_{14}Cl_2N_2O$	1-Chloroacetyl)-3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole	333	81	68
3A		$C_{18}H_{16}Cl_2N_2O_2$	1-(Chloroacetyl)-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole	363	130	68
4A		$C_{17}H_{14}ClN_3O_3$	1-(Chloroacetyl)-5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	343	160	70
5A		$C_{18}H_{17}ClN_2O_2$	1-(Chloroacetyl)-5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	328	124	75

Table No.4 Physical Properties of the Acetylated Pyrazoline derivatives

Compd Code.	Structure	Mol. Formula	IUPAC Name	Mol. Wt.	M.P	% Yield
6A		$C_{17}H_{14}ClN_3O_3$	1-Acetyl-3-(4-chlorophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1*H*-pyrazole	343	260	75
7A		$C_{17}H_{15}ClN_2O$	1-Acetyl-3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole	298	140	75

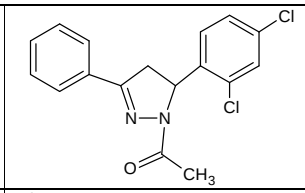
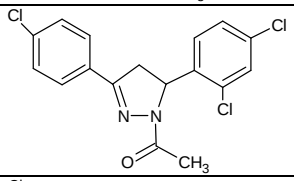
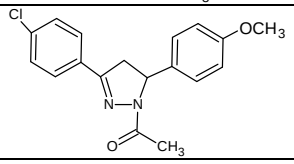
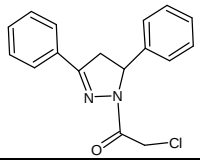
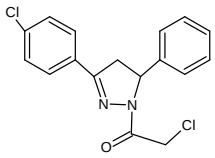
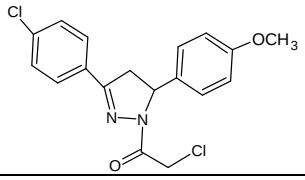
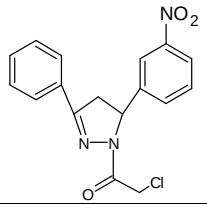
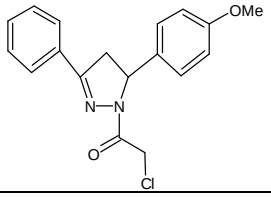
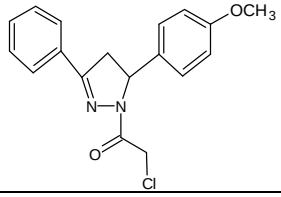
8A		$C_{17}H_{14}Cl_2N_2O$	1-Acetyl-5-(2,4-dichlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	333	125	70
9A		$C_{17}H_{13}Cl_3N_2O$	1-Acetyl-3-(4-chlorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole	367	115	70
10A		$C_{18}H_{17}ClN_2O_2$	1-Acetyl-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole	328	135	75

Table No.5 Antibacterial activity of the compounds synthesized.

Compd Code.	Structure	Mol. Formula	IUPAC Name	Zone of Inhibition(mm)			
				1*	2*	3*	4*
1A		$C_{17}H_{15}ClN_2O$	1-Chloroacetyl)-3,5-diphenyl-4,5-dihydro-1*H*-pyrazole	16	11	14	13
2A		$C_{17}H_{14}Cl_2N_2O$	1-Chloroacetyl)-3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole	12	12	12	12
3A		$C_{18}H_{16}Cl_2N_2O_2$	1-(Chloroacetyl)-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole	14	12	14	13
4A		$C_{17}H_{14}ClN_3O_3$	1-(Chloroacetyl)-5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole	17	12	11	13

5A		$C_{18}H_{17}ClN_2O_2$	1-(Chloroacetyl)-5-(4-methoxyphenyl) -3-phenyl-4,5-dihydro-1*H*-pyrazole	16	12	12	13
6A		$C_{17}H_{14}ClN_3O_3$	1-Acetyl-3-(4-chlorophenyl)-5-(3-nitrophenyl)- 4,5-dihydro-1*H*-pyrazole	12	11	10	13

Antimicrobial activity

All the synthesised compounds were screened for antibacterial activity against S.aureus, S.epidermis, P.aerouginosa and E.coli by agar diffusion method at the concentration of 100µg/ml in DMSO using Penicillin G. as standard for antibacterial activity [5,6]. The zone of inhibition was measured in mm and the activity was compared with standard.

RESULTS AND DISCUSSION

The titled compounds were synthesized according to the procedures as given in the experimental section. The reactions were monitored by TLC. The physical constants like melting point and solubility were determined for all the intermediate and final products. The compounds were further characterized by IR and H^1 NMR. All the titled compounds were evaluated for their antibacterial activities.

Table 5: Indicates the anti-microbial activity of the synthesised compounds.
The test compounds were screened for anti-microbial activity and the following activity was observed.

Staphylococcus aureus (Gram-positive)
1A, 4A, 5A showed maximum activity of all the compounds tested, were 2A, 6A, 8A, 10A has minimal activity and others 3A, 7A,9A were moderately active.

Staphylococcus epidermis (Gram-positive)
None of the compounds tested showed maximum activity, all the compounds were having the zone of inhibition at less than 12mm, hence this organism was found to be more resistant to the test compounds.

Escherichia coli (Gram-negative)
1A, and 3A were having maximum inhibition, 10A, 9A, 6A, and 4A showed least activity.

Pseudomonas aeruginosa (Gram-negative)
All the ten compounds were having almost similar activity with the zone of inhibition between 11-13mm against this bacteria.

Thus it was found that the chloroacetylated pyrazoline derivatives were more effective against all the organisms tested, than acetylated pyrazoline derivatives.

The results indicate that presence of a chloroacetyl group attached to N of pyrazoline ring is optimum for antibacterial properties.

CONCLUSION

- The main focus of this research work was to synthesize, purify, characterize and evaluate antimicrobial activities of the newly synthesized pyrazoline derivatives.
- A series of titled compounds, i.e., [1A-10A] have been synthesized.
- The yield of the synthesized compounds was found to be in range from 65%- 75%.
- Structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and ^{1}H NMR.
- A single method was used to perform the antibacterial activities using the agar diffusion method.
- Most of the compounds tested showed good antibacterial activities at the concentration of 100 µg/0.1ml using Benzyl penicillin as standard.
- The compounds were tested against four species of bacteria namely, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermis* Among the synthesized compounds, derivative 4A, 1Aand 5A showed strong antibacterial activity against *Staph.Aureus*, the remaining compounds exhibited mild to moderate activity.
- In case of *E.coli*, 1A and 3A exhibited considerable activity, while the others showed moderate activity.
- All the synthesized compounds had mild antibacterial effect against *Staph. Epidermi*s and *P.aeruginosa*
- Hence, newly synthesized pyrazoline derivatives do possess considerable Antibacterial activity and further lead optimization should be carried out for the better-expected antibacterial activity.

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