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Synthesis and Biological Activities of Some Novel 2-Pyrazolines from Chalcones

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ABSTRACT

Chalcones have been reported to present various biological activities such as anti-inflammatory, antioxidant, antitubercular, antibacterial activities. It is a basic moiety of many heterocyclic systems containing oxygen, sulphur and nitrogen. Nitrogen containing heterocyclic derivatives synthesized from Chalcones have exhibited anti-inflammatory, anticancer and antimicrobial activities. An attempt has been made to synthesize Chalcones by the reaction of 3-acetyl-2,5-dimethylfuran with various aromatic and heteroaromatic aldehydes. Further, Chalcones derivatives were cyclized to 2-pyrazoline analogs by phenyl hydrazine in absolute ethanol in the presence of pyridine. The newly synthesized Pyrazoline derivatives have been characterized by IR, ¹HNMR, Mass spectra and elemental analysis and evaluated for their analgesic, anti-inflammatory, antifungal and antibacterial activities. It was found that compounds having 3,4,5-trimethoxyphenyl ring and 4-methoxyphenyl ring at the 5-position of the 2-pyrazoline ring possessed the maximum analgesic activity, moreover it has also exhibited good antifungal and antibacterial activities.

Keywords: Chalcones, 2-Pyrazolines, analgesic activity, antifungal activity, antibacterial activity.

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INTRODUCTION

Chalcones are unique templates that are associated with several biological activities and are well known intermediates for synthesizing various heterocyclic compounds. They are secondary metabolites of terrestrial plants, precursors for the biosynthesis of flavonoids. The introduction of a halogen into the benzenoid part of these α,β -unsaturated ketones enhances their biological activity. Nitrogen containing heterocyclic play an important role in medicinal chemistry . A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry. Pyrazole is a π -excessive aromatic monocyclic heterocyclic containing two nitrogen atoms in a five membered 1,2-diazole ring.

The Pyrazoline nucleus is a ubiquitous feature of various compounds possessing many pharmacological and physiological activities and they are useful materials in drug research. It was reported in the literature that different substituted 2-pyrazolines possess antimicrobial, anti-inflammatory, analgesic, antipyretic, antidepressant, antitubercular, antiamoebic, anthelmintic, anticonvulsant, antihypertensive, anti diabetic, antitumor, anti-HIV, local anesthetic, antioxidant, insecticidal and tranquilizing activities.

MATERIALS AND METHOD

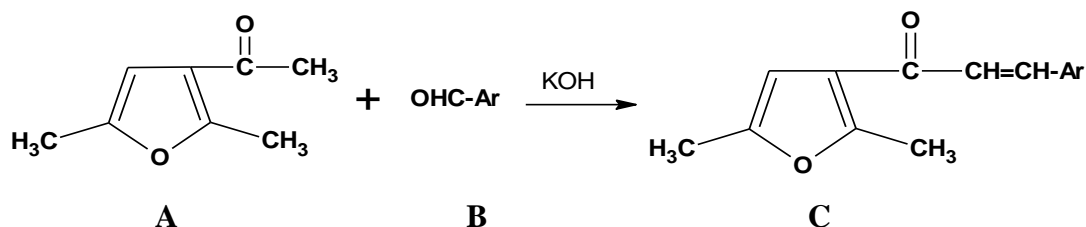
All the melting points were determined in a Boitus melting point apparatus and are uncorrected. The ^1H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

The mass spectra of the compounds were recorded either on Agilent 1100 ESI-Mass (Turbo Spray) Spectrophotometer or API-ES mass spectrometer using positive mode ionization method. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution using n-hexane, mixtures of hexane and ethyl acetate (5%, 10%, 15 %, 25%, 50% and 75% hexane in ethyl acetate), ethyl acetate and mixtures of ethyl acetate and methanol (1%, 2%, 5% and 10% ethyl acetate in methanol). Fractions each of 100 mL were collected. The separations of the compounds were checked on TLC under UV lamp and also by spraying the plates with 10% sulphuric acid. Elemental analyses were carried out with a Perkin-Elmer model 2400 series II apparatus.

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation:

Equimolar quantities (0.005 mol) of 3-acetyl-2,5-dimethylfuran and respective aldehydes were mixed and dissolved in minimum amount of alcohol. To this, aqueous potassium hydroxide solution (50%, 7.5 mL) was added slowly and mixed occasionally for 24 h, at

room temperature. Completion of the reaction was identified by TLC using silica gel-G. After completion of the reaction, the mixture was poured onto crushed ice, acidified if necessary with dilute hydrochloric acid, and the solid that separated was isolated by filtration, dried and purified by column chromatography on silica gel with a mixture of ethyl acetate and hexane as the mobile phase. The overall reaction involving the formation of chalcones are shown in **Scheme 1**

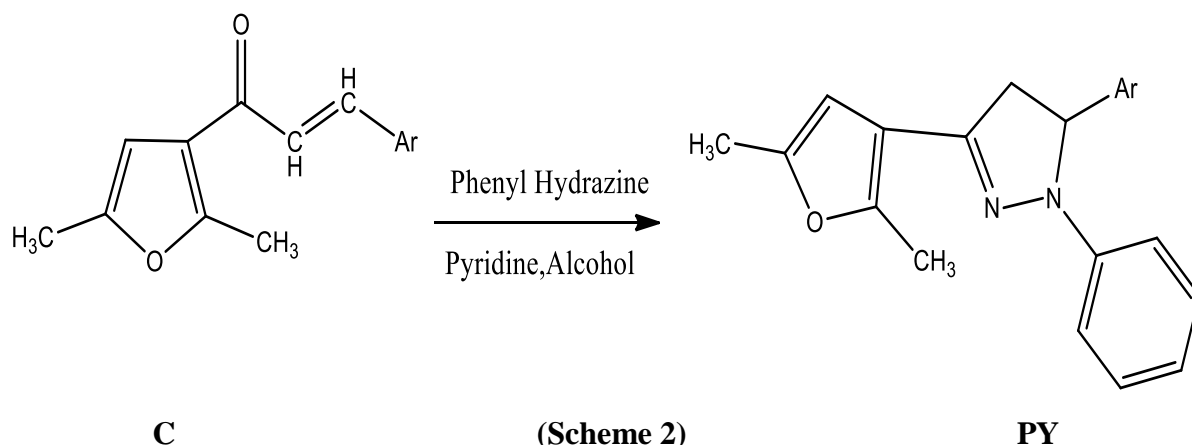


A = 3-acetyl-2,5-dimethylfuran; B = aldehydes; C = 1-(2',5'-dimethyl-3'-furyl)-3-(aryl)-2-propen-1-one

General procedure for the synthesis of 2-Pyrazolines:

The chalcones were condensed with phenyl hydrazine in absolute ethanol in the presence of pyridine at reflux temperature (2 to 6 hrs). The solvent was completely evaporated and the residue was poured into ice cold water, which resulted in the formation of the corresponding 2-pyrazolines (Scheme 2). Reaction completion was identified by TLC using silica gel - G. After completion of the reaction, the reaction mixture was poured into crushed ice with constant stirring. The separated solid was filtered and dried. It was purified by column chromatography on silica gel, using ethyl acetate and hexane mixture as the mobile phase. After purification, the 2-pyrazolines were obtained as light or bright colored powders.

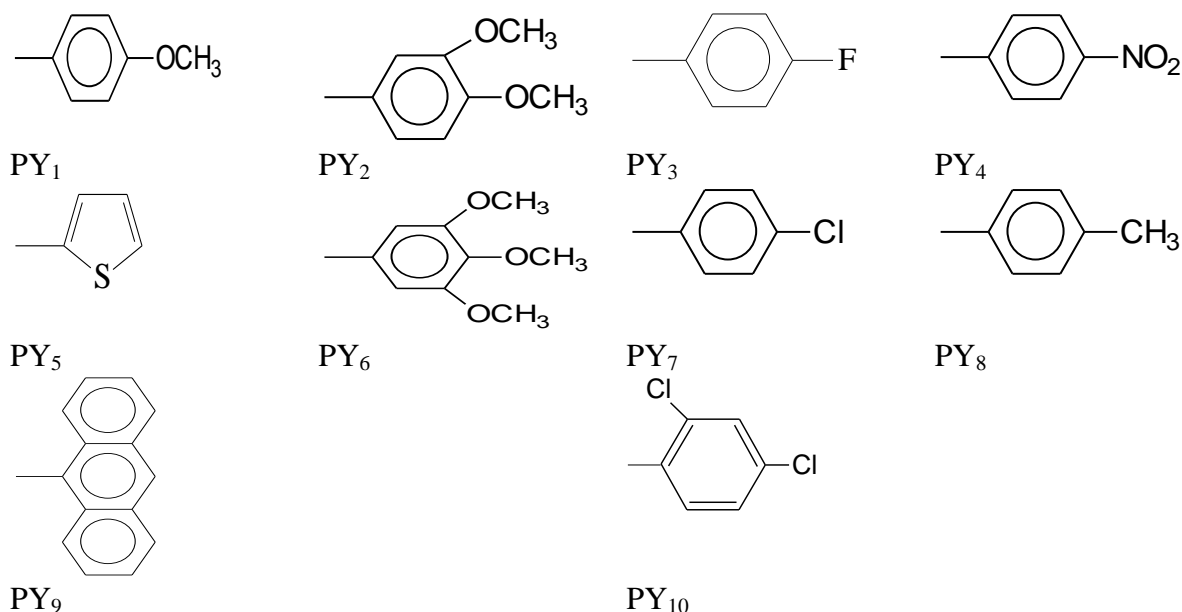
Scheme 2 :



C = 1-(2',5'-dimethyl-3'-furyl)-3-(aryl)-2-propen-1-one;

PY = 1-phenyl-3-(2',5'-dimethyl-3'-furyl)-5-(aryl)-2-pyrazoline

Ar



List of synthesized 3,5 disubstituted 2- Pyrazolines compounds:

- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''-methoxy phenyl) -2-pyrazoline: (PY₁)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(3'',4''-dimethoxy phenyl) -2-pyrazoline: (PY₂)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''-fluoro phenyl) -2-pyrazoline: (PY₃)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''-nitro phenyl) -2-pyrazoline: (PY₄)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(2''-thienyl) -2-pyrazoline: (PY₅)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(3'',4'',5''-trimethoxy phenyl) -2-pyrazoline:(PY₆)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''chloro phenyl) -2-pyrazoline:(PY₇)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''-methyl phenyl) -2-pyrazoline:(PY₈)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl)- 5-(9''-anthryl) -2-pyrazoline: (PY₉)
- 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(2'',4''-dichloro phenyl) -2-pyrazoline:(PY₁₀)

RESULTS AND DISCUSSION

Spectral properties of 2-pyrazolines: The 2-pyrazolines showed the characteristic H_A, H_B and H_X protons at δ 3.05, 3.78 and 5.28 respectively as doublet of doublets (dd) with $J_{AB} = 16.5$ Hz, $J_{AX} = 7.30$ Hz and $J_{BX} = 9.5$ Hz.

Antimicrobial studies of 2-Pyrazolines:

Antibacterial activity:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5mL) to give a concentration of 1000 μ g/mL. Benzyl penicillin solution was prepared to give a concentration of 1000 μ g/mL in sterilized distilled water. All the compounds were tested at dose levels of 50 μ g (0.05 mL) and 100 μ g (0.1mL) and DMSO used as a control. The solutions of each test

compound, control and reference standards (0.05 and 0.1 mL) were added separately in the cups and the plates were kept undisturbed for at least 2 h in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1 °C for 24 h. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

Among all the compounds tested, compounds **PY₃**, **PY₇** and **PY₁₀** possessed maximum activity. All these three compounds possessed the halogens on the aromatic ring and thus reveal the positive contribution of electron withdrawing groups to the antibacterial activity, which is consistent with the literature reports. Compounds having these substituent's on the heteryl ring can also be synthesized and screened for antibacterial activity, with a hope to get better compounds in this series. A QSAR study on a large data bank of 2-pyrazolines may further provide insights into the structural requirements and the contributing physico-chemical properties in enhancing the antibacterial activity.

Antifungal activity of 2-Pyrazolines:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 mL) to give a concentration of 1000 µg/mL. Fluconazole solution was also prepared at a concentration of 1000 µg/mL in sterilized distilled water. All the compounds were tested at dose levels of 50 µg (0.05 mL) and 100 µg (0.1 mL) and DMSO used as a control. The solutions of each test compound, control and reference standards (0.05 and 0.1 mL) were added separately in the cups and the plates were kept undisturbed for at least 2 hr in a refrigerator to allow diffusion of the solution properly into the PDA medium. Petri dishes were subsequently kept at room temperature for 48 h. After that, the diameter of zone of inhibition in mm surrounding each of the cups was measured with the help of an antibiotic zone reader.

Among all the compounds tested, compounds **PY₇** and **PY₁₀** produced maximum inhibitory zones. Compounds with electron withdrawing groups enhanced the activity. Compounds having electron releasing groups also contributed favorably to the antifungal activity. The contributing physico-chemical properties of these compounds, however, need to be established by QSAR studies. 2-Pyrazolines having electron withdrawing and releasing substituent's on the heteryl ring can also be synthesized and screened for antifungal activity in order to get compounds with promising activity.

Table 1: Physical characterization data of synthesized 2- Pyrazolines(PY₁-PY₁₀)

Compound	Molecular formula	Relative molecular mass (RMM)	Melting point (°C)	Yield (%)
PY ₁	C ₂₂ H ₂₂ N ₂ O ₂	346	106-108	67
PY ₂	C ₂₃ H ₂₄ N ₂ O ₃	376	145-147	72
PY ₃	C ₂₁ H ₁₉ FN ₂ O	334	96-98	78
PY ₄	C ₂₁ H ₁₉ N ₃ O ₃	361	157-159	75
PY ₅	C ₁₉ H ₁₈ N ₂ OS	322	115-117	67
PY ₆	C ₂₄ H ₂₆ N ₂ O ₄	406	134-136	69
PY ₇	C ₂₁ H ₁₉ ClN ₂ O	350	121-123	75
PY ₈	C ₂₂ H ₂₂ N ₂ O	330	92-94	71
PY ₉	C ₂₉ H ₂₄ N ₂ O	416	223-225	72
PY ₁₀	C ₂₁ H ₁₈ Cl ₂ N ₂ O	384	89-91	69

Table 2: Elemental Analysis data of 2-Pyrazolines(PY₁-PY₁₀)

Compound	(% Calculated value)			(% practically found)		
	C	H	N	C	H	N
PY ₁	76.28	6.40	8.09	76.23	6.38	8.17
PY ₂	73.38	6.43	7.44	73.28	6.39	7.46
PY ₃	75.43	5.73	8.38	75.41	5.71	8.29
PY ₄	69.79	5.30	11.63	69.74	5.27	11.72
PY ₅	70.78	5.63	8.69	70.72	5.63	8.58
PY ₆	70.92	6.45	6.89	70.87	6.45	6.94
PY ₇	71.89	5.46	7.98	71.85	5.42	7.95
PY ₈	79.97	6.71	8.48	79.96	6.68	8.56
PY ₉	83.63	5.81	6.73	83.58	5.76	6.84
PY ₁₀	65.46	4.71	7.27	65.41	4.68	7.36

Table 3: IR spectral data (KBr disc) of 2- Pyrazolines(PY₁-PY₁₀)

Compound	Position of absorption band (cm ⁻¹)
PY ₁	1598 (C=N), 1503 (C=C), 1225 (C-O-C), 1157 (O-CH ₃) and 1122 (C-N)
PY ₂	1573 (C=N), 1565 (C=C), 1233 (C-O-C), 1185 (O-CH ₃) and 1133 (C-N)
PY ₃	1598 (C=N), 1566 (C=C), 1119 (C-F), 1071 (C-O-C) and 1148 (C-N)
PY ₄	1583 (C=N), 1563 (C=C), 1541 (N=O, asymmetric), 1325 (N=O, symmetric), 1105 (C-N) and 1055 (C-O-C)
PY ₅	1597 (C=N), 1573 (C=C), 1125 (C-N), 1063 (C-O-C) and 675 (C-S)
PY ₆	1598 (C=N), 1504 (C=C), 1223 (C-O-C), 1160 (O-CH ₃) and 1125 (C-N)
PY ₇	1596 (C=N), 1553 (C=C), 1123 (C-N), 1055 (C-O-C) and 855 (C-Cl)
PY ₈	1597 (C=N), 1524 (C=C), 1106 (C-N) and 1058 (C-O-C)
PY ₉	1593 (C=N), 1563 (C=C), 1110 (C-N) and 1063 (C-O-C)
PY ₁₀	1595 (C=N), 1553 (C=C), 1121 (C-N), 1065 (C-O-C) and 863 (C-Cl)

Table 4: ^1H NMR spectral data (400MHz) of 2-Pyrazolines (PY₁-PY₁₀)

Compound	Chemical shift (δ) in ppm
PY ₁	3.05 (1H, dd, H _A), 3.78 (1H, dd, H _B), 5.28 (1H, dd, H _X), 3.75 (3H, s, OCH ₃), 6.45-7.22 (10H, Ar-H), 2.2(3H,s,Ar-CH ₃), 2.5(3H,s, Ar-CH ₃).
PY ₂	3.1 (1H, dd, H _A), 3.69 (1H, dd, H _B), 5.18(1H, dd, H _X), 3.75 (3H, s, OCH ₃), 3.85 (3H,s,OCH ₃), 6.35-7.49 (9H,Ar-H), 2.3(3H,s, Ar-CH ₃), 2.6 (3H,s, Ar-CH ₃).
PY ₃	3.05 (1H, dd, H _A), 3.78 (1H, dd, H _B), 5.22(1H, dd, H _X), 6.44-7.47 (10H, Ar-H), 2.18(3H,s, Ar-CH ₃), 2.5(3H,s, Ar-CH ₃).
PY ₄	3.08 (1H, dd, H _A), 3.80 (1H, dd, H _B), 5.27 (1H, dd, H _X), 6.75-7.66 (10 H, Ar-H), 2.2(3H,s, Ar-CH ₃), 2.6(3H,s, Ar-CH ₃).
PY ₅	3.18 (1H, dd, H _A), 3.77 (1H, dd, H _B), 5.46 (1H, dd, H _X), 6.45-7.48 (9H, Ar-H), 2.2(3H,s, Ar-CH ₃), 2.5(3H,s, Ar-CH ₃).
PY ₆	3.11 (1H, dd, H _A), 3.72 (1H, dd, H _B), 5.12 (1H, dd, H _X), 3.79 (6H, s, 2 x OCH ₃), 3.82 (3H, s, OCH ₃), 6.45-7.49 (8H , Ar-H), 2.25(3H,s, Ar-CH ₃), 2.55(3H,s, Ar-CH ₃).
PY ₇	3.02 (1H, dd, H _A), 3.75 (1H, dd, H _B), 5.17(1H, dd, H _X), 6.41-7.45 (10H, Ar-H), 2.5(3H,s, Ar-CH ₃), 2.65(3H,s, Ar-CH ₃).
PY ₈	3.10 (1H, dd, H _A), 3.68 (1H, dd, H _B), 5.20 (1H, dd, H _X), 2.29 (3H, s, Ar-CH ₃), 6.41-7.46 (10H, Ar-H), 2.3(3H,s, Ar-CH ₃), 2.55(3H,s, Ar-CH ₃).
PY ₉	3.53 (1H, dd, H _A), 3.89(1H, dd, H _B), 5.51 (1H, dd, H _X), 6.50-8.47 (15 H, Ar-H), 2.45(3H,s, Ar-CH ₃), 2.8(3H,s, Ar-CH ₃).
PY ₁₀	3.02 (1H, dd, H _A), 3.67 (1H, dd, H _B), 5.17 (1H, dd, H _X) and 6.41-7.45 (9H, Ar-H), 2.2(3H,s, Ar-CH ₃), 2.6(3H,s, Ar-CH ₃).

Table 5: Results of Antibacterial activity of 2-Pyrazolines (PY₁-PY₁₀)

Compound	Ar	Zone of inhibition (in mm) Quantity in µg/mL									
		<i>B. subtilis</i>		<i>B. pumilis</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>P. vulgaris</i>	
		50	100	50	100	50	100	50	100	50	100
PY ₁	4''-methoxy phenyl	16	21	15	19	15	18	18	21	16	17
PY ₂	3'',4''-dimethoxy phenyl	21	27	21	25	21	11	21	24	23	25
PY ₃	4''-fluoro phenyl	23	25	21	26	20	23	22	24	21	26
PY ₄	4''-nitro phenyl	17	21	18	22	20	23	18	21	18	22
PY ₅	2''-thienyl	20	25	18	24	21	26	19	25	19	24
PY ₆	3'',4'',5''-trimethoxy phenyl-	18	21	16	18	13	16	15	21	18	20
PY ₇	4''chloro phenyl	19	23	21	26	22	24	20	24	21	26
PY ₈	4''-methyl phenyl	19	26	18	25	21	23	20	25	18	23
PY ₉	9''-anthryl	21	27	20	26	19	23	20	25	19	22
PY ₁₀	2'',4''-dichloro phenyl	23	25	22	27	20	23	21	26	25	26
Benzyl penicillin		26	28	25	28	24	27	24	27	26	27

Table 6: Results of Antifungal activity of 2-Pyrazolines (PY₁-PY₁₀)

Compound	Ar	Zone of inhibition (in mm) Quantity in µg/mL					
		<i>A. niger</i>		<i>C. albicans</i>		<i>R. oryzae</i>	
		50	100	50	100	50	100
PY ₁	4''-methoxy phenyl	14	16	18	20	15	19
PY ₂	3'',4''-dimethoxy phenyl	21	24	22	25	20	23
PY ₃	4''-fluoro phenyl	20	23	19	23	20	23
PY ₄	4''-nitro phenyl	19	24	19	26	18	25
PY ₅	2''-thienyl	18	25	20	24	23	27
PY ₆	3'',4'',5''-trimethoxy phenyl-	18	20	18	18	13	16
PY ₇	4''chloro phenyl	21	25	20	25	18	25
PY ₈	4''-methyl phenyl	15	19	17	20	19	22
PY ₉	9''-anthryl	21	26	20	24	24	27
PY ₁₀	2'',4''-dichloro phenyl	22	25	21	26	20	25
Fluconazole		24	27	23	28	21	27

CONCLUSION:

Chalcones derivatives were cyclised using phenyl hydrazine in absolute ethanol in the presence of pyridine to obtain 2- Pyrazoline derivatives. All the 2-pyrazoline derivatives were evaluated for analgesic, antibacterial and anti fungal activities. Compounds 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(4''chloro phenyl) -2-pyrazoline (PY₇)and 1- phenyl -3-(2',5'-dimethyl-3'-furyl) -5-(2'',4''-dichloro phenyl) -2-pyrazoline (PY₁₀) were exhibited excellent antibacterial and antifungal activity. Compounds which carries 4-chloro phenyl, 2,4-dichloro phenyl substituent's at Pyrazol ring exhibited maximum antibacterial and antifungal activity.

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