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Synthesis and Biological Evaluation of Some Novel 3-Methyl Pyrazol-5-One Derivatives as Potential Antimicrobial Agents

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ABSTRACT

In the present research work, the motto was to develop new chemical entities as potential antimicrobial agent. The recent expansion of antimicrobial drug research has occurred because there is a critical need for new antimicrobial agents to treat these life threatening invasive infections. In the present study three series of new substituted pyrazolone derivatives (3i, 7a-l) were synthesized by the condensation reaction of pyrazolones with various substituted aromatic aldehyde (5), further intermediate reacts with a benzyl chloride. These newly synthesized compounds were characterized by IR spectra. New compounds were screened for their antimicrobial studies against *S. aureus, B. subtilis, E. coli and P.aeruginosa, C.albicans, A.niger.* The results revealed that compounds substituent showed significant antibacterial activity against all tested microorganisms and fungi as compared to the standard drug Streptomycin, fluconazole.

Keywords: 3-methyl pyrazol-5-one, pyrazolone, antimicrobial

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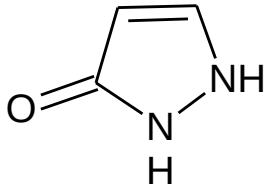
INTRODUCTION

Pyrazolone nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. The similar compounds synthesized through different routes bear variable magnitudes of biological activities. The knowledge of various synthetic pathways and the diverse physicochemical parameters of such compounds draw the especial attention of medicinal chemists to produce combinatorial library and carry out exhaustive efforts in the search of lead molecules. There are vast numbers of pharmacologically active heterocyclic compounds many of which are in regular clinical use [1]. The investigational approaches towards Structure- Activity Relationship focusing the search of optimized candidates have become immensely important. The present research provides a broad view of the synthesis and properties of compounds having pyrazolone nucleus.

Pyrazolone belongs to a class of heterocyclic compounds containing a five membered ring made up of two nitrogen and one keto group as heteroatom with the formula $C_3H_4N_2O$[2,3]



Pyrazolone is an important pharmacophore which exhibits widespread pharmacological properties, such as anticancer [4,5], analgesic, anti-inflammatory, antipyretic [6,7,8], antioxidant [9,10,11], antiproliferative [12] myocardial ischemia [13], treatment of fatal neurogenerative diseases [14], cardiovascular diseases [15] antimicrobial [16-21],and hypoglycemic [22].

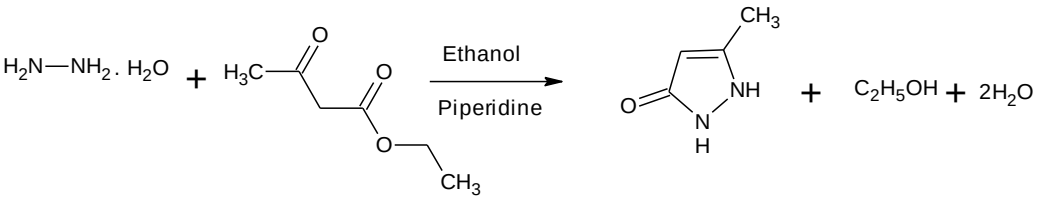
MATERIALS AND METHODS

The melting points were determined by open capillary method and are uncorrected. UV spectras were recorded on Thermo scientific spectrum 2600 spectrophotometer. The IR spectras were recorded on a Jasco FT-IR spectrophotometer, model-4100 by using KBr disc method.^{1}H and ^{13}C spectras were recorded on a Bruker AVANCE 400 MHz spectrometer at 25°C using tetramethylsilane as an internal standard and DMSO-δ6 as the solvent. The chemical shifts are expressed in δ ppm. Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as visualizing agent. All chemicals used were of RESEARCH-LAB FINE CHEM INDUSTRIES, Mumbai. Solvents and Chemicals were purified wherever required.

Synthesis [23,24]

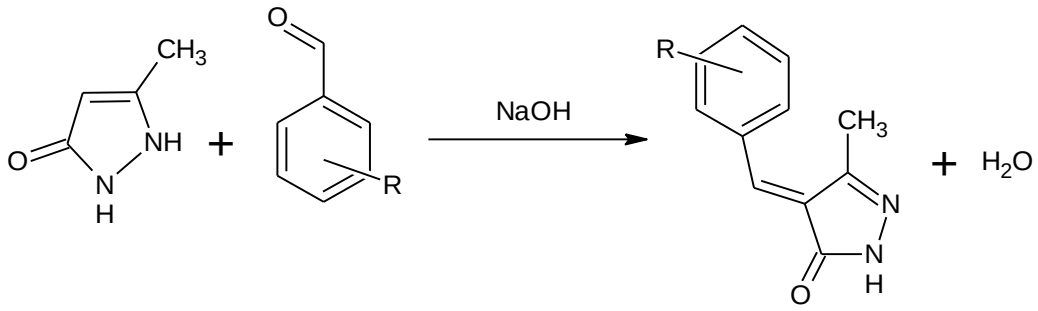
Synthetic Procedure:

Step- 1



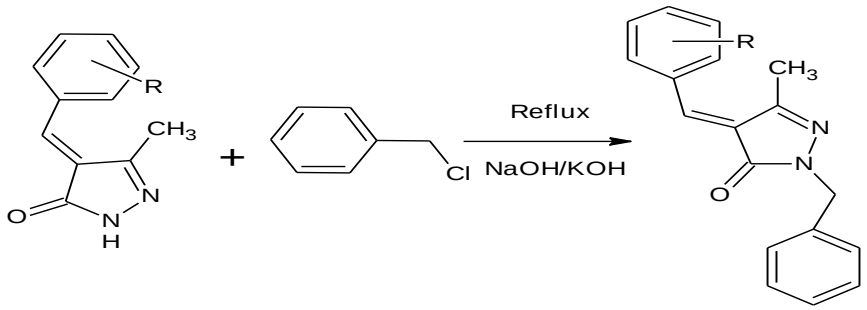
1 2 3i

Step-2



3i 4 5

Step-3



5 6 7(a-f)

General procedure for Synthesis of 3-methyl pyrazol-5-one (3i)

65 g (0.5 mole) of ethyl acetoacetate was taken in a 250 ml conical flask and stirred magnetically during slow drop wise addition of a solution of 25 g (0.5mole) of hydrazine hydrate in 40 ml absolute ethanol. The temperature of the reaction mixture increased during reaction so that temperature was regulated at 60°C. The reaction mixture was cooled in an ice bath to complete the crystallization. After standing for some time for completion of crystallization, it was filtered and the solid was washed with cold alcohol, dried and used for further step.

General procedure for Synthesis of 3-methyl pyrazol-5-one derivatives (5)

Pyrazolone (0.01 mole) was taken in a 100 ml of freshly prepared 20% sodium hydroxide

alcoholic solution was poured into it. The mixture was stirred with magnetic stirrer for 30min. After that substituted aromatic and aliphatic aldehyde (0.01 moles) was added to the reaction mixture and kept under stirring for 8 hrs. The reaction mixture was transferred into crushed ice and neutralized with dilute hydrochloric acid to precipitate the product. It was filtered, dried and purified by recrystalization from ethanol.

General procedure for Synthesis of 3-methyl pyrazol-5-one derivatives by Benzylation reaction 7(a-f)

To a solution of intermediate (0.003 mole) in absolute ethanol (30 ml), 1.12 gm KOH/ NaOH and 0.03 mole of benzyl chloride added. The mixture refluxed for 2 hrs. After cooling precipitate was collected by filtration and dried and recrystallised from water. Similarly, other compounds were synthesized with some change in reflux time and reaction work up. The physical data of the compounds are given in table .1

TLC solvent: a = n-Hexane : Ethyl acetate (7:3),

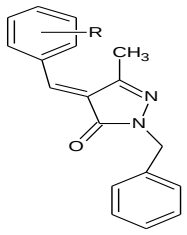
b = Ethyl acetate : Benzene (5:5),

c = n-hexane

Recrystalisation solvent: Hot Water

Melting Point: 3: 222-223°C, 7(a-f): > 260°C

Table 1: Physical Characterization of synthesized compound (7a-l)



Sr. No	Compound code	-R	Molecular formula	Molecular Weight	TLC Solvent	% Yield
1	3i	-	$C_4H_6N_2O$	98.103	a	80
2	7a	- H	$C_{18}H_{16}N_2O$	276.33	b	58.48
3	7b	-OCH_3	$C_{19}H_{18}N_2O_2$	306.35	c	49.21
4	7c	*o*-Cl	$C_{18}H_{15}N_2OCl$	310.75	b	45.68
5	7d	m-Cl	$C_{18}H_{15}N_2OCl$	310.75	b	49.56
6	7e	*p*-Cl	$C_{18}H_{15}N_2OCl$	310.75	b	51.16
7	7f	*p*-OH	$C_{18}H_{17}N_2O_2$	293	b	48.64
8	7g	*p*-$N(CH_3)_2$	$C_{20}H_{21}N_3O$	319.40	b	85.61
9	7h	*m*-NO_2	$C_{18}H_{16}N_3O_3$	321.33	b	53.42
10	7i	*p*-OH, m-OCH_3	$C_{18}H_{17}N_2O_2$	322.35	b	49.51
11	7j	*o*-OH	$C_{18}H_{17}N_2O_2$	293	b	56.23
12	7k	*p*-Br	$C_{18}H_{15}N_2OBr$	355.22	c	50.16
13	7l	C_7H_6	$C_{20}H_{18}N_2O$	302.36	b	43.89

Characterizations [25-29]

FT-IR Spectroscopy

Compound code 3i 5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one

Assignment: FTIR (KBr, cm^{-1}): 1614 (carbonyl C=O), 1552 (C=N), 1447 (C-CH_3), 3470 (N-H stretch)**.**

Compound code 7a (4*Z*)-2-benzyl-4-benzylidene-5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one **Assignment:** FTIR (KBr, cm^{-1}): 1740 (carbonyl C=O stretch), 1626 (C=N stretch), 1463 (C-C stretch), 1578 (C=C stretch).

Compound code 7b (4*Z*)-2-benzyl-4-(4-methoxybenzylidene)-5-methyl-2,4-dihydro 3*H*-pyrazol-3-one. **Assignments:** FTIR (KBr, cm^{-1}): 1730 (carbonyl C=O stretch), 1612 (C=N stretch), 1457 (C-C stretch), 1513 (C=C stretch).

Compound code 7c (4*Z*)-2-benzyl-4-(2-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one. **Assignment:** FTIR (KBr, cm^{-1}): 1717(carbonyl C=O stretch), 1603 (C=N stretch), 1433 (C-C stretch), 749 (C-Cl stretch).

Compound code 7d (4*Z*)-2-benzyl-4-(3-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one. **Assignment:** FTIR (KBr, cm^{-1}): 1751 (carbonyl C=O stretch), 1619 (C=N stretch), 1385 (C-C stretch), 1519 (C=C stretch), 3060 (Ar-CH stretch), 703 (C-Cl), 2920 (Aliphatic CH stretch).

Compound code 7e (4*Z*)-2-benzyl-4-(4-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one. **Assignment:** FTIR (KBr, cm^{-1}): 1658 (carbonyl C=O stretch), 1602 (C=N stretch), 1438 (C- CH_3 stretch), 789 (C-Cl).

Compound code 7f (4*Z*)-2-benzyl-4-(4-hydroxybenzylidene)-5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one. **Assignment:** FTIR (KBr, cm^{-1}): 1759 (carbonyl C=O stretch), 1610 (C=N stretch), 1381 (C- C Stretch), 1503 (C=C stretch), 1245 (-OH bend).

Evaluation of Anti-microbial Activity:

A. Antibacterial activity

Broth dilution method

Broth dilution method is also known as tube dilution method. It is quantitative method for determining the minimum inhibitory concentration (MIC) of the antibiotic against bacteria to be tested. In this method, serial dilutions of the antibiotics are taken in test tubes and a standardized suspension of the bacterium is inoculated. After incubating overnight, the MIC of the antibiotics is determined by observing the lowest concentration of antibiotics that inhibits growth of bacteria. The minimum bactericidal concentration (MBC) can be estimated by this method by

subculturing from the lowest concentration of drug that kills bacteria.

Nutrient Agar for Bacteria:

Accurately weighed nutrient agar (2.8 g) dissolved in distilled water (50 ml). Autoclave the solution (for 15 min. at 15 lbs), cool the solution and transferred it in sterile test tubes in sterile area. Keep the slant at 37°C at inclined position.

Preparation of Nutrient broth:

13 gm of nutrient broth was dissolved in 1000 ml distilled water.

Table.2: Composition of Nutrient Broth:

Ingredients Grams/liter	
Peptic digest of animal tissue	5
Sodium Chloride	5
Beef extract	1.5
Yeast extract	1.5
Distilled water q.s.	1000

The above prepared nutrient broth was sterilized by autoclave at 121°c for 15 minutes at 15-lbs/in^2 pressure.

Preparation of fungal suspension

Gram positive bacteria (*Escherichia coli, Pseudomonas aeruginosa*), Gram negative bacteria (*staphylococcus aureus, bacillus subtilis*) 0.9 % saline solution by sterile inoculation loop.

Observations:

At the end of incubation period the tubes were examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of compound present in the medium.

B. Antifungal activity

Sabouraud's broth

For the evaluation of antifungal activity Sabouraud's medium was used. Low pH and high sugar concentration makes this media selective for the fungi than bacteria. Glucose and peptone were dissolved in water with heating, cooled and the pH was adjusted to 5.4 with HCl and filtered. Total matrix was sterilized at 120°C for 15minutes.

Table .3: Composition of Sabouraud's broth

Sr. No	Ingredient	Quantity
1	Glucose	40 g
2	Peptone	10 g
3	Water q.s to	1000 ml

Preparation of fungal suspension

Fungal spores of *Aspergillus niger* and *Candida albicans* were diluted in 5ml of 0.9 % saline solution by sterile inoculation loop.

Preparation of test solution

10 mg of the test compound was dissolved in 10 ml of DMSO. From this 1 ml of the solution was taken and diluted to 10 ml with DMSO. Now, the concentration of the test compound is 100µg/ml.

Preparation of standard solution

The standard drug used in this testing is streptomycin, Fluconazole respectively.

Procedure

The stock solution of (100 µg/ml) of compounds was prepared in DMSO. To each tube containing sterilized Sabouraud's liquid medium (5 ml),Nutrient broth medium (5ml), 5 ml of drug solution was added. The serial dilutions were made to obtain concentrations (in mg/ml) such as 500, 250, 125, and 62.5. Each tube was inoculated with the microorganism and was incubated to 30°C for 14 days. Positive control tubes (organism + broth + DMSO) and negative control tubes (broth + drug) were also prepared. The readings were taken and expressed as (-), if inhibition of growth is seen and (+), if inhibition of growth is not seen [11,30-33].

RESULTS AND DISCUSSION:

The antimicrobial screenings of 13 synthesized compounds were undertaken using broth dilution method (MIC method). Table 4 lists the screening results of the tested compounds **(3,7a-l)** against Gram positive bacteria (*Escherichia coli, Pseudomonas aeruginosa),* Gram negative bacteria (*Staphylococcus aureus, Bacillus subtilis*) and fungal strains (Candida albicans, Aspergilus niger). The obtained data revealed that most of the compounds showed moderate activities against the microorganisms used at dose 31.25-250 µg/ml, using minimum inhibitory concentration method. Streptomycin was used as standard antibacterial and fluconazole as standard antifungal. DMSO was used as a blank exhibited no activity against any of the used organisms. From the result it is also clear that the compounds tested showed variable toxicity against different microorganisms. The variation in toxicity can be attributed to different structures and functional groups attached to the basic nucleus.

It was observed that compounds **7c, 7e, 7k** were active against *E. Coli* and compounds **7e, 7k** were active against *Staphylococcus aureus* and compound **7c, 7k** were active against *Bacillus subtilis.* Also compounds **7e** and **7k** were found active against *Candida albicans*. It is well noticed that compounds **7c**, **7e** and **7k** showed considerable good activity against all the tested microorganisms with MIC value 31.25µg/ml. It was revealed that different substituent's led to different antimicrobial activity. Therefore, structure activity relationship in the compounds

demonstrated that compounds with electron withdrawing substituent's (**7c, 7e, 7k**) showed more potent activity against microorganisms than those with electron donating substituent's (**7b, 7d, 7f, 7g, 7h**). However, the antimicrobial potency of all compounds was less than that of standard used, streptomycin, fluconazole.

Table.4: **Antimicrobial activity**

Compound Code	MIC in µg/ml E. Coli	S. aureus	P. aeruginosa	B. subtilis	*C. albicans*	*A. niger*
3i	125	125	125	125	62.5	125
7a	125	62.5	125	125	250	250
7b	250	125	125	125	125	250
7c	31.25	62.5	62.5	31.25	62.5	62.5
7d	62.5	62.5	62.5	125	125	62.5
7e	31.25	31.25	62.5	62.5	31.25	31.25
7f	250	250	250	125	250	250
7g	125	125	250	250	250	125
7h	125	250	125	250	250	125
7i	62.5	62.5	125	125	125	125
7j	125	125	250	125	250	250
7k	31.25	31.25	62.5	31.25	31.25	62.5
7l	62.5	62.5	62.5	31.25	125	125
Std.	**3.90**	**3.90**	**3.90**	**3.90**	**3.90**	**3.90**

CONCLUSION:

The compounds were screened for antibacterial activity against two gram positive and gram negative bacteria and antifungal screening also done against two fungi. Synthesized pyrazolones exhibited moderate to good antimicrobial activity.

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