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SYNTHESIS OF PYRAZOLONE DERIVATIVES BY USING HYDRAZINE ANALOGS, CHARACTERIZATION AND ITS ANTIFUNGAL ACTIVITY

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ABSTRACT

A series of 1-substituted analogues of 3-methyl pyrazol-5-one derivatives are synthesized by using some hydrazine substituted analogs through the condensation & ring closure reaction with ethylacetoacetate.The synthesized pyrazoles are subjected to analyse for physical data and IR spectral studies. The IR spectral interpretation results are correlated satisfactory for the above synthesized compounds. They were evaluated for in-vitro anti-fungal activity by agar well diffusion method in potato dextrose agar medium using standard drug clotrimazole 100µg concentration against Aspergillus niger (ATCC 16404) microbial strains.The pyrazoles compounds were tested at 250µg, 500µg and 1000 µg concentrations. Among this 3a, 3b, and 3c are sensitive, compound 3d shows less potency and 3e is not sensitive against Aspergillus niger.

Keywords: Pyrazoles, Pyrazolones, Agar well diffusion method, MIC, Antifungal and Aspergillus niger.

INTRODUCTION

Medicinal chemistry is the discipline concerned with the design, development and synthesis of pharmaceutical drugs [1-3]. It involves the creation and refinement of molecules for the purpose of creating or improving drugs. It is grounded in synthetic organic chemistry, a discipline in which scientists combine small molecules to create new ones.Nitrogen-linked heterocyclic compounds have recently played a significant role in medicinal and pesticidal activities. Pyrazole is a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions as represented by the molecular formula C3H4N2. [4]

Pyrazole derivatives exhibit a wide range of biological activities .The literature survey reveals that previous research article shows pyrazole derivatives have antibacterial activity , antifungal activity [5-7] , antidiabetic [8], anti-inflammatory activity [9,10], COX-2 inhibitors antimalarial activity [11], antimicrobial, anticancer activity [12]. Based on this, our work we are aimed to design and characterize derivatives of pyrazole [13] with high potency and least toxicity antimicrobial agent. We are planning to synthesize certain 1-substituted analogues of 3-methyl pyrazol-5-one derivatives by using ethyl acetoacetate reaction with hydrazine derivatives. The pyrazoles are synthesized by using some hydrazine substituted molecules through the condensation & ring closure reaction followed by the above synthesized compounds are analyzed for physical properties & I.R spectral studies. Finally we decided to evaluate in-vitro anti-fungal activity [19] of the synthesized pyrazolones using Aspergillus niger (ATCC 16404) microbial strains.

EXPERIMENTAL

SYNTHESIS OF 3-METHYL 1-SUBSTITUTED -1, 2-DIHYDRO PYRAZOL-5-ONEs

PROCEDURE

Mix together 0.384 mol of redistilled ethyl acetoacetate (1) and 0.37 mol of hydrazine derivatives chemically named hydrazine (2a), phenyl hydrazine (2b) ,thiosemicarbazide (2c) , 2,4-dinitrophenylhydrazine (2d) and isoniazid (2e) in a large evaporating dish separately.

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Heat mixtures on a boiling water bath in cupboard for about 2 hrs and stir from time to time using glass rod. Allow heavy reddish syrup to cool somewhat about 10 ml of ether and stir mixture vigorously. The syrup which is insoluble in ether, will solidify in within 15 minutes and allowed to stand for 24 hrs., Filter solid at pump and wash it thoroughly with ether to remove colored impurities. Recrystallize it from a equal volume of ethanol and water. The yield of 3-methyl-1-substituted -1, 2-dihydro pyrazol-5-ones (3a-e) recorded were ranged from 56 to 83 % and interpreted by I.R spectrum. [14].

IR Spectra Interpretation of Compound-3b

N-H Stretching (2^0 amine) : 3360.69 cm^{-1}, N-H Bending (2^0 amine) : 1497.17 cm^{-1}, N-H Bending (2^0 amide) : 1540.65 cm^{-1}, C=O : 1657.13 cm^{-1}, C-N Stretching : 1348.96 cm^{-1}, CH3 Aliphatic : 1461.75 cm^{-1}, C-H Stretching (SP3) : 2968.01 cm^{-1} , C-H Bending (SP3) : 1348.96 cm^{-1}, =C-H Stretching : 3090.90 cm^{-1}, =C-H Bending : 841.64 cm^{-1} , C-C Stretching : 1255.42 cm^{-1} , Aromatic C-H Bending : 789.97 cm^{-1} , Aromatic C=C Stretching : 1572.79 cm^{-1}

IR Spectra Interpretation of Compound-3c

N-H Stretching (2^0 amine) : 3370.73 cm^{-1}, N-H Bending (2^0 amine) : 1499.83 cm^{-1}, N-H Stretching (2^0 amide) : 3428.59 cm^{-1}, C=O : 1640.11 cm^{-1} , C-N Stretching : 1334.85 cm^{-1} , C-H Bending (SP3) : 1364.18 cm^{-1} , C=C Aliphatic : 1593.25 cm^{-1} , =C-H Stretching : 2995.66 cm^{-1} , =C-H Bending : 871.26 cm^{-1} , C-C Stretching : 1274.02 cm^{-1}, C=S Stretching : 1185.75 cm^{-1}

ANTIFUNGAL ACTIVITY
AGAR- WELL DIFFUSION METHOD

PRINCIPLE

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.Clotrimazole (standard antifungal agent, concentration: 100µg, Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%, *Aspergillus niger* (ATCC 16404*)*

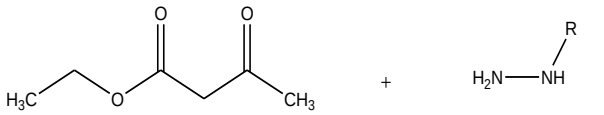
PROCEDURE

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg, 500µg and 1000µg were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).[19]

Table 1: Nature, Solubility and chemical name of synthesized pyrazolones

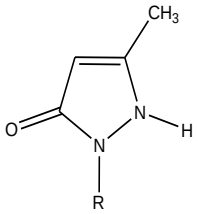
Synthesized pyrazoles	Chemical name	Colour	Nature	Solubility
3a	3-methyl-1,2-dihydropyrazol-5-one	Dull white	Slightly amorphous solid powder	Soluble in Water Partially soluble in Dimethylsulfoxide Partially soluble in Alcohol
3b	3-methyl-1-phenyl-1,2-dihydro pyrazol-5-one	Reddish brown	Semisolid	Soluble in Water Soluble in Dimethylsulfoxide Soluble in Alcohol
3c	3-methyl-5-oxo-2H-pyrazole-1(5H)-carbothioamide	Reddish pink	Solid powder	Soluble in Water Soluble in Dimethylsulfoxide Soluble in Alcohol
3d	1-(2,4-dinitrophenyl)-3-methyl-1,2-dihydropyrazol-5-one	Brownish orange	Solid powder	Soluble in Water Soluble in Dimethylsulfoxide Soluble in Alcohol
3e	1-isonicotinoyl-3-methyl-1,2-dihydropyrazol-5-one	Orange white	Solid powder	Soluble in Water Soluble in Dimethylsulfoxide Soluble in Alcohol

Scheme



Ethyl acetoacetate (1)

Hydrazine (2a-e)



3-methyl-1-substituted-1,2-dihydropyrazol-5-one (3a-e)

3a-R=H, 3b-R= C_6H_5, 3c-R=CS-NH_2, 3d-R=2,4-dinitrophenyl,3e-R=CO-C_5H_4N

Table 2: Antifungal Activity of Compound - 3a

Sample	Concentration (µg)	Zone of inhibition (mm)
3a	Clotrimazole(100µg)	18
	250	13
	500	16
	1000	23

Table 3: Antifungal Activity of Compound -3b

Sample	Concentration (µg)	Zone of inhibition (mm)
3b	Clotrimazole(100µg)	18
	250	12
	500	17
	1000	21

Table 4: Antifungal Activity of Compound -3c

Sample	Concentration (µg)	Zone of inhibition (mm)
3c	Clotrimazole(100µg)	19
	250	12
	500	16
	1000	19

Table 5: Antifungal Activity of Compound -3d.

Sample	Concentration (µg)	Zone of inhibition (mm)
3d	Clotrimazole(100µg)	17
	250	Nil

	500	Nil
	1000	13

Table 6: Antifungal Activity of Compound -3e

Sample	Concentration (µg)	Zone of inhibition (mm)
3e	Clotrimazole(100µg)	18
	250	Nil
	500	Nil
	1000	Nil

Figure 1: IR Spectrum of Compound-3b

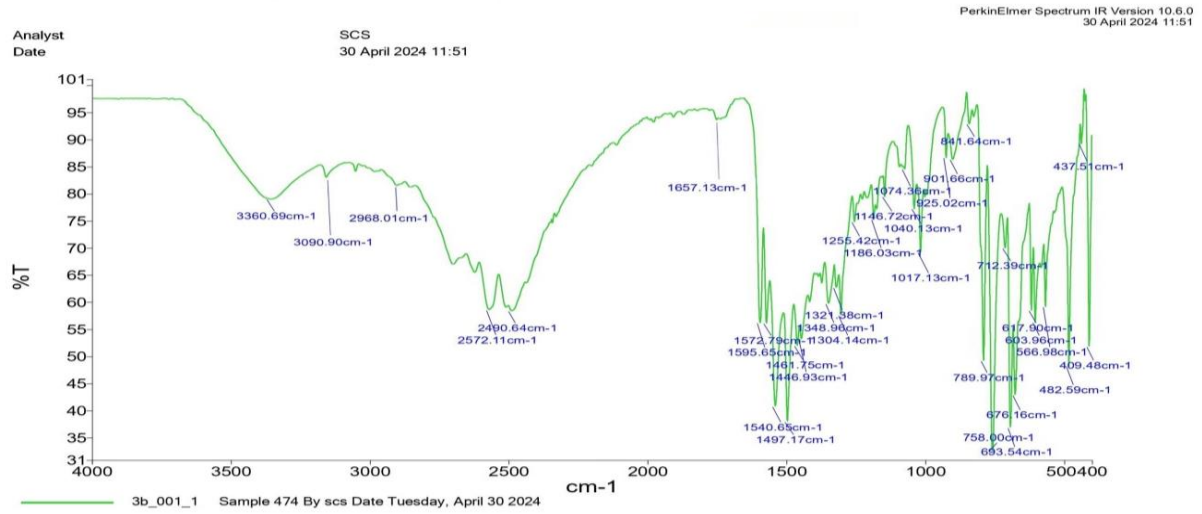


Figure 2: IR Spectrum of Compound-3c

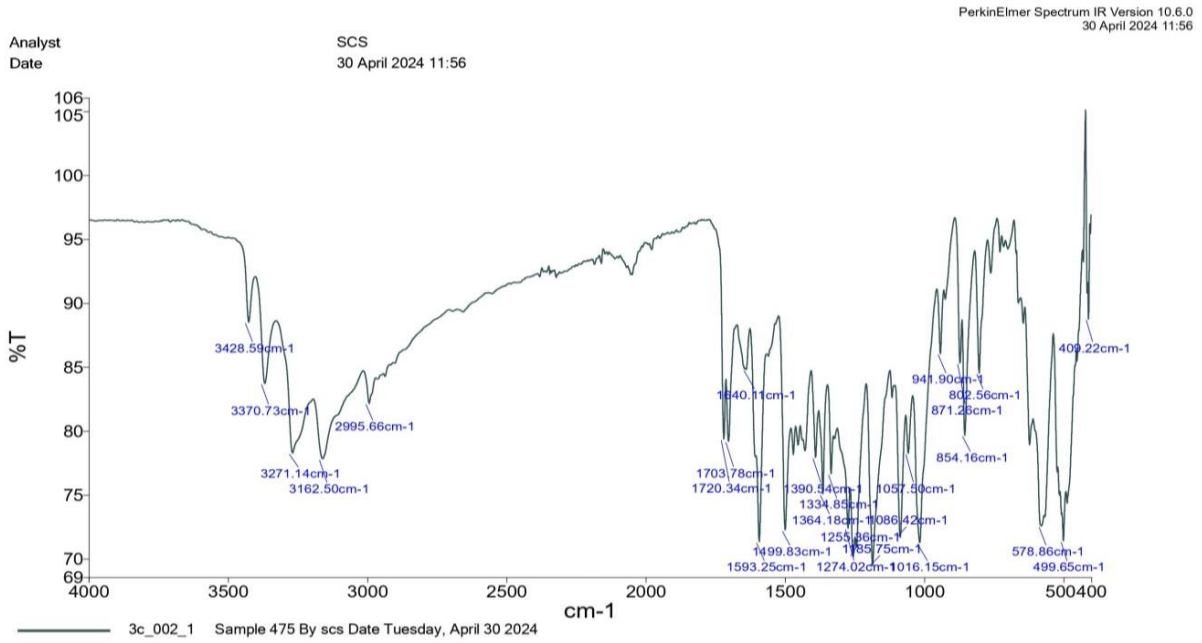


Figure 3: Antifungal Activity of Compound - 3a

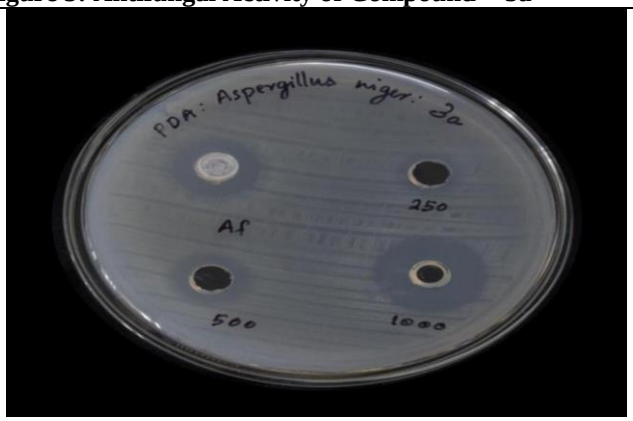


Figure 4: Antifungal Activity of Compound - 3b

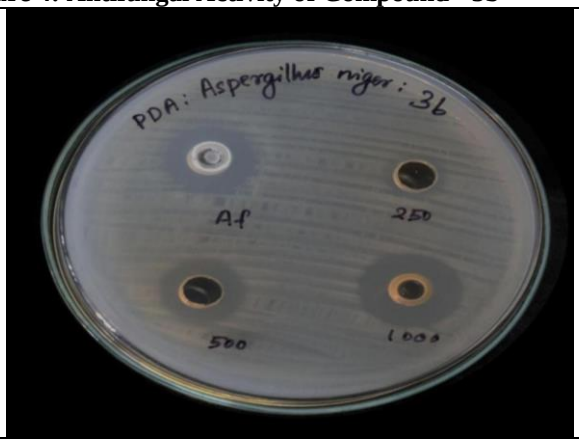


Figure 5: Antifungal Activity of Compound - 3c

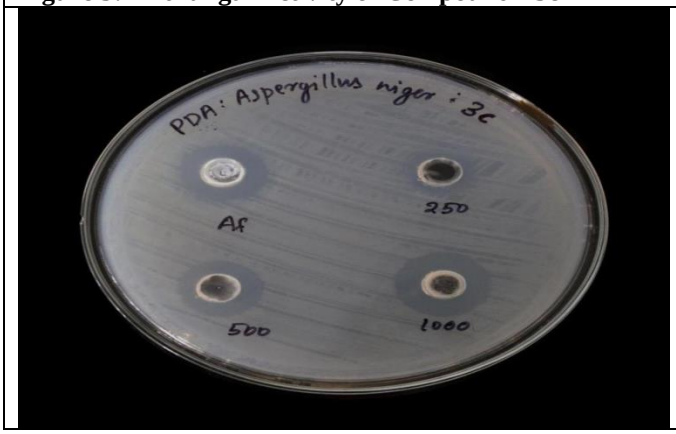


Figure 6: Antifungal Activity of Compound - 3d

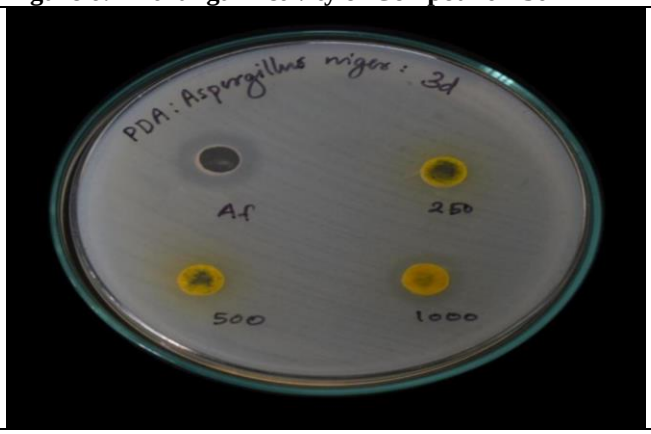


Figure 7: Antifungal Activity of Compound - 3e

RESULTS AND DISCUSSION

The synthesized pyrazoles yeild getting around 56-83% .All the synthesized compounds were characterized by IR spectroscopy and physical analysis.The IR spectrum of the compounds are correlated with respective fragments and functional groups. Infra-red studies of the compounds shows that C−H stretching aromatic ranging from 2995.56 cm^{-1}- 3105.90 cm^{-1}N-H

stretching ranging from 3593.84 cm^{-1}- 3360.69 cm^{-1}, C=O stretching ranging from 1640.11 cm^{-1}- 1723.67 cm^{-1}, and C-N stretching ranging from 1117.33 cm^{-1}- 1348 cm^{-1}[15-18].The antifungal activity of synthesized pyrazoles against *Aspergillus niger* (ATCC 16404*)* are displayed in the tables 2 to 6 and figures 3to7.

The tested pyrazoles compound 3a,3b,3c are sensitive against *Aspergillus niger* showed considerable zone of inhibition.Among this 3d shows less potency and 3e is not sensitive against *Aspergillus niger*.Anyway compounds 3a,3b and 3c exhibited moderate antifungal activity results against *Aspergillus niger* higher concentrations at 250µg, 500µg and 1000 µg as MIC.The compound 3d is sensitive at higher concentration 1000 µg.The compound 3e failed to unveils antifungal activity against *Aspergillus niger*

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