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SYNTHESIS AND ANTIFUNGAL ACTIVITY OF NOVEL PYRAZOLE DERIVATIVES

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Abstract: The reaction of substituted aromatic aldehydes with acetophenone using 20% potassium hydroxide in ethanol to form intermediate. Second, synthesis of compounds was carried out by semithiacarbezide and above intermediate. 3-Phenyl 5-substituted-4, 5-dihydro-1H-pyrazole-1-carbothioamide 2(a-d). Purity was checked by TLC and the chemical structures of synthesized compounds were elucidated by their IR, ^{1}H NMR analysis data. The synthesized compounds were screened for Antifungal activity.

Keywords*:* Aldehyde, Acetophenone, Chalcones, *Pyrazole,* IR, ^{1}H-NMR, Antifungal activity.

Introduction:

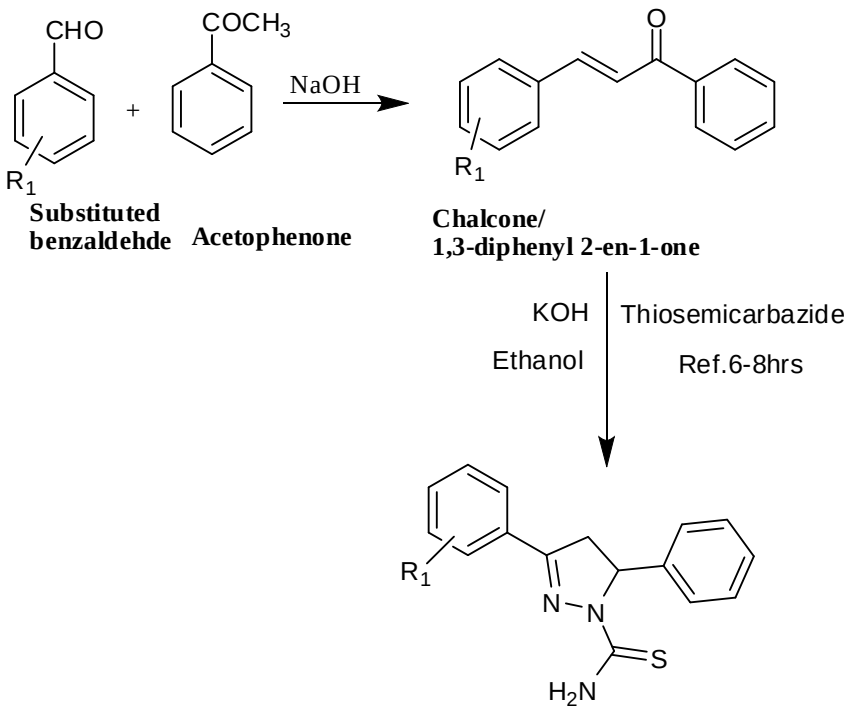
Hetero aromatic compounds impart considerable attention in the design of biologically active molecules and advanced organic materials[1]. The pyrazole ring is common in a number of biologically active molecules. More recently extensive studies have been focused on pyrazole derivatives for exhibiting analgesic[2], anti-inflammatory[3], anticonvulsant[4], antidepressant[5], antiulcer[6], antidiabetic[7], cytotoxic[8], antitubercular[9] and antibacterial[10]. Antidepressants and anticonvulsants are among the most widely utilized drugs for the treatment of CNS disorders. Considerable interest has been focused on thepyrazole structure, which has been known to possess a broad spectrum of biological activities. Since Candida albicans (C.albicans) and Aspergillus fumigatus (A. fumigatus) are the main causative fungi in which Candida albicans has been identified as the major opportunistic pathogen in the etiology of fungal infections; however, the frequency of other Candida species is increasing dramatically . Current antifungal therapy suffers from drug-resistant strains of pathogenic bacteria towards available antibiotics and many drugs have become resistant to routine antifungal drugs. Therefore, there is urgent need for development of new drugs alternative as well as effective drugs with higher efficiency, broader spectrum, and lower toxicity. A number of antifungal azoles were discovered in the last three decades and are introduced in clinical practice up till now. Despite some significant advances in this field, there is a continuing increase in the incidence of fungal infections, together with a gradual rise in azole resistance. However clinical use of azoles is limited because of an increase of resistant strains, particularly during the long-term treatment[10] Bacterial infections are the second acknowledged cause of death worldwide and the third in developed countries. The therapeutic efficiency of antimicrobials has become more complex due to the emergence of multidrug resistance[11]. *B. monosperma* has numerous pharmacological activities such as anthelmintic, anti-conceptive, anticonvulsive, antidiabetic, antidiarrheal, antiestrogenic and antifertility, anti-inflammatory, antifungal, antibacterial, antistress, anticancer, antioxidant, chemopreventive, haemagglutinating,

hepatoprotective, thyroid inhibitory, antiperoxidative, hypoglycemic effects, wound healing activities, anti-giardiasis, antifertility, chemo preventive activities and radical scavenging activities [12]

Materials and Methods:

Melting points of all the synthesized compounds were determined by open capillary tubes using paraffin bath and are uncorrected. The homogeneity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: acetone (2:1) as developer detected by iodine vapors. The IR spectra were recorded on a JASCO FT-IR 4100 spectrophotometer, using KBr powder technique. ^{1}H NMR spectra were recorded on a Varian-NMR-mercury 300 MHz spectrophotometer in $CDCl_3$ using TMS as an internal standard [13,14]

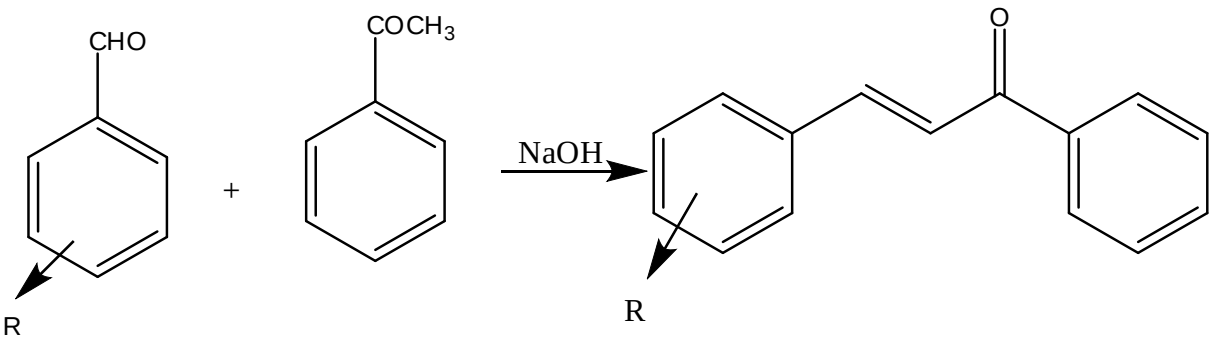
Scheme of synthesis:



3-phenyl,5-substituted phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide

Experimental:

1. **Synthesis of chalcones or 1-phenyl-3 substituted phenyl propene 1-one**



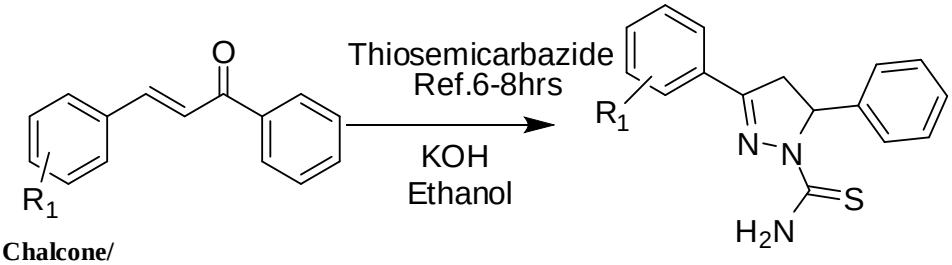
Substituted benzaldehde | Substituted Acetophenone | Chalcone/ 1,3-diphenyl 2-en-1-one

A solution of 10% NaOH and rectified spirit was taken in Erlenmeyer flask provided with mechanical stirrer. The flask was immersed in a bath of crushed ice, acetophenone (0.83ml, 0.43mol) was poured and stirring was started, substituted aromatic aldehydes (0.43mol) was then added. Temperature of the mixture was kept within 15 to 30°C. Stirring was continued until the mixture becomes so thick that stirring is no longer effective and then reaction mixture was left in a refrigerator overnight. The product was filtered, washed with cold water until the washing are neutral to litmus and recrystallized from methanol. This substance should be handled with great care[15]

1a IR(KBr) cm^{-1}:1634(C=O); 1152(Cl); 1190(NH-C=C).

1a^{1}HNMR (CDCl) ‰ : 8.24, 8.30 (e,2H, 5H,3H), 8.0, 8.05(e, 2H, 2H, 6H), 7.78 7.82 (a, 2H, 6íH, 2íH), 7.6-7.68 (b, 2H, 5íH, 2íH), 7.5-7.56 (c, 3H, 3íH, 4íH, 5íH).

(2) Synthesis of 3-Phenyl 5-substituted -4, 5-dihydro-1H-pyrazole-1- carbothioamide



Chalcone/ 1,3-diphenyl 2-en-1-one | **3-phenyl,5-substituted phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide**

A mixture of corresponding chalcones (0.001 mol), thiosemicarbazide (0.001 mol), and KOH (0.0025 mol) was refluxed in ethanol (25 -30 mL) for 4-8 hrs. The solution was poured into ice-water. The precipitate was filtered and crystallized from methanol [16,17]

2a. IR (KBr): 3354(N-H), 3134 (unsaturated aromatic C=C), 1700(C=S), 2855 (CH_3 Asymmetric), 1455(C-N).

^{1}H NMR: δ 7.4-5.5 (Aromatic ring); 7.30-7.22(nitro phenyl), and 2.01(d, 6H, CH_3); 5.9 (OH): 12.3 (N-H)

2b:,IR (KBr): 3750 (N-H stretching),2500 (C-H stretching), 1800 (C=C stretching (Aromatic), 1590(C=N stretching) , 1700(C=S stretching),

2c: IR (KBr): 3800 (N-H stretching),2400 (C-H stretching), 1800 (C=C stretching (Aromatic), 1500(C=N stretching) , 1650(C=S stretching), 660 (C-Cl)

^{1}H NMR: δ 7.5-6.5 (Aromatic ring); 7.26-7.31(Chloro phenyl), and 2.01(d, 6H, CH_3); 5.8 (OH):N-H(12.3)

2d: 3720 (N-H stretching),2770 (C-H stretching), 2000 (C=C stretching (Aromatic), 1900(C=N stretching), 1390(NO_2), 1337 (C-N stretching),1012(C=S stretching), 660 (C-NO_2)

Results and Discussion:

Table No. 1. Physical data of synthesized 3-Phenyl.5-substituted-4,5-dihydro-1H-pyrazole-1-carbothioamide derivatives

Compound Code	R	Molecular formula	Molecular weight	Melting point	Yield (%)	RF
2a	H	$C_{10}H_9N_3S$	203	128-120	70.25	0.6
2b	Ar	$C_{15}H_{14}N_3S$	268	120-122	74.33	0.7
2c	4-Chloro-Ar	$C_{15}H_{13}N_3SCl$	302	228-230	82.85	0.7
2d	4-Nitr-Ar	$C_{15}H_{13}N_4SO_2$	313	180-182	78.71	0.6

Physiochemical Characterization of Synthesized Compounds

Physiochemical characterizations of synthesized compounds were performed by using of various parameters such as melting point, solubilities, R_f value etc.

Solubility Prameters:

The solubility of synthesized compounds was determined in various polar and nonpolar solvents.

Table No. 2. Solubility of synthesized compounds

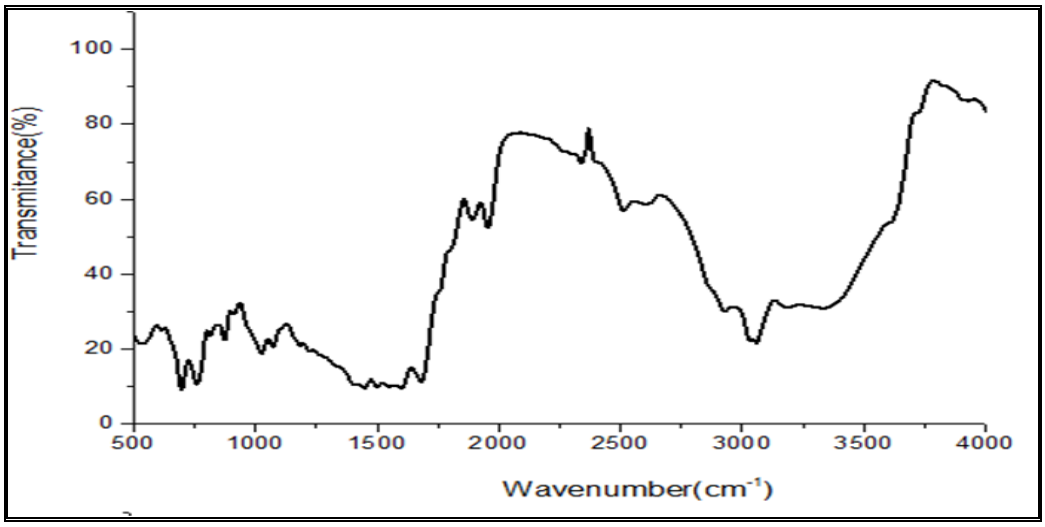
Solvent Comp. →	Cold Water	Methanol	Acetone	Ethyl acetate	Chloroform	Dioxane	Benzene	n-hexane
2a	-	++	++	++	+++	+	++	-
2b	-	++	++	++	+++	+	++	-
2c	-	++	++	++	+++	+	++	-

2d	-	++	++	++	+++	+	++	-

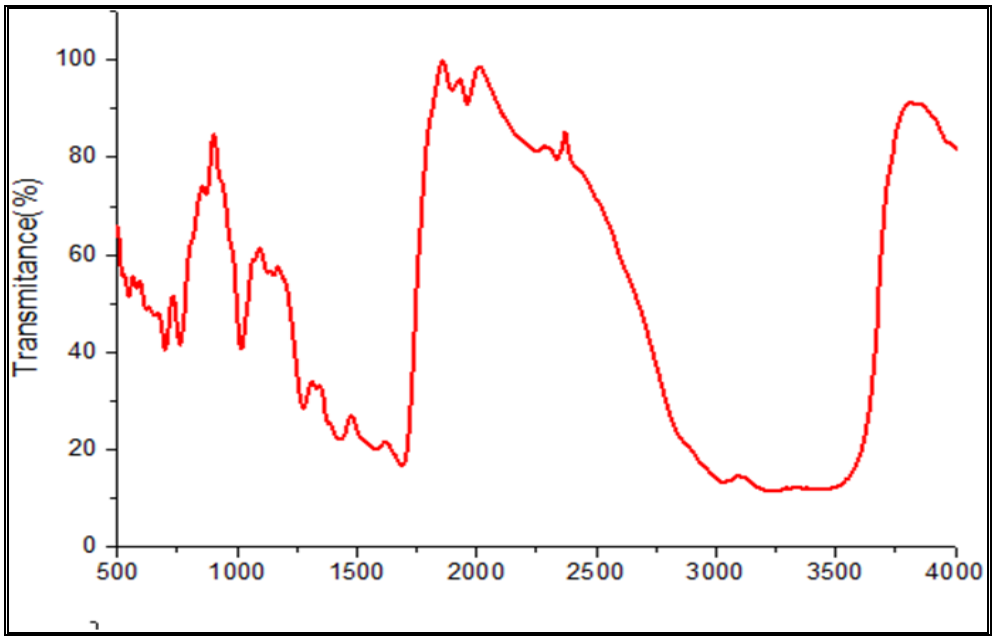
Thin layer chromatography (TLC):

The thin layer chromatography was performed of the synthesized compounds. Silica gel G was used as stationary phase. In TLC, we found single spot some time with tailing with different R_f values for each compound. This single spot indicates the purity of synthesized compounds.

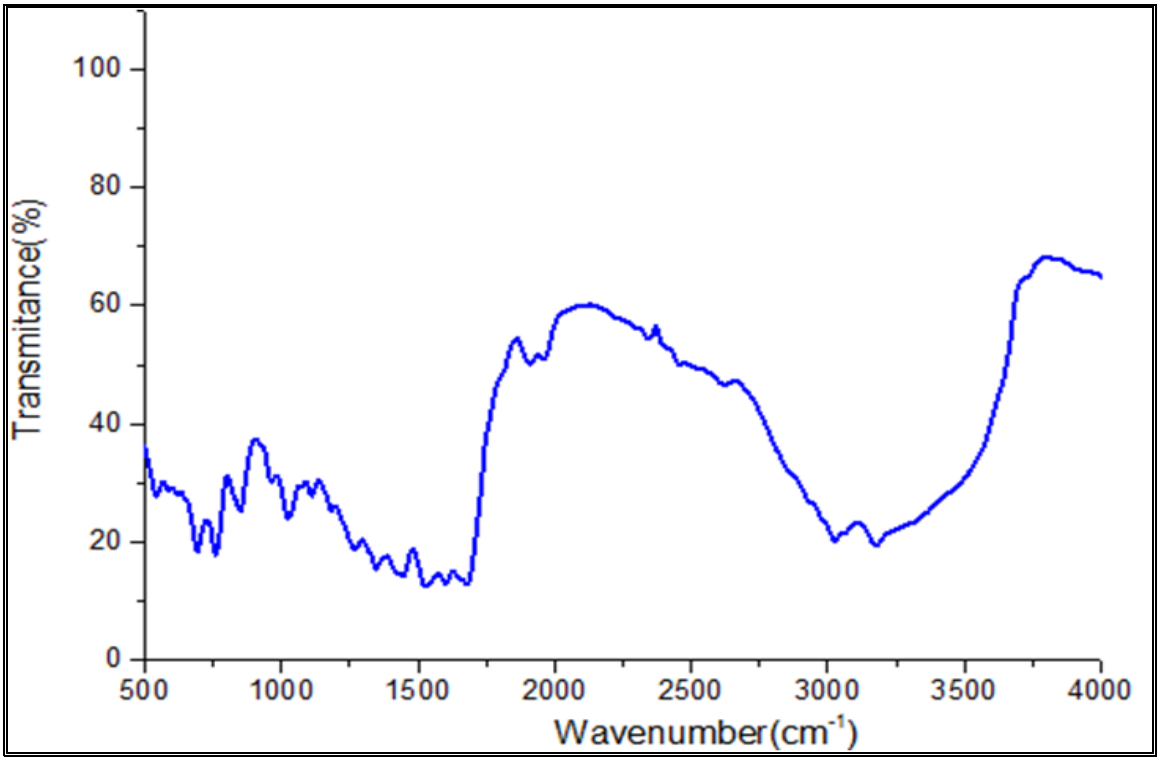
IR Interpretation: 2a



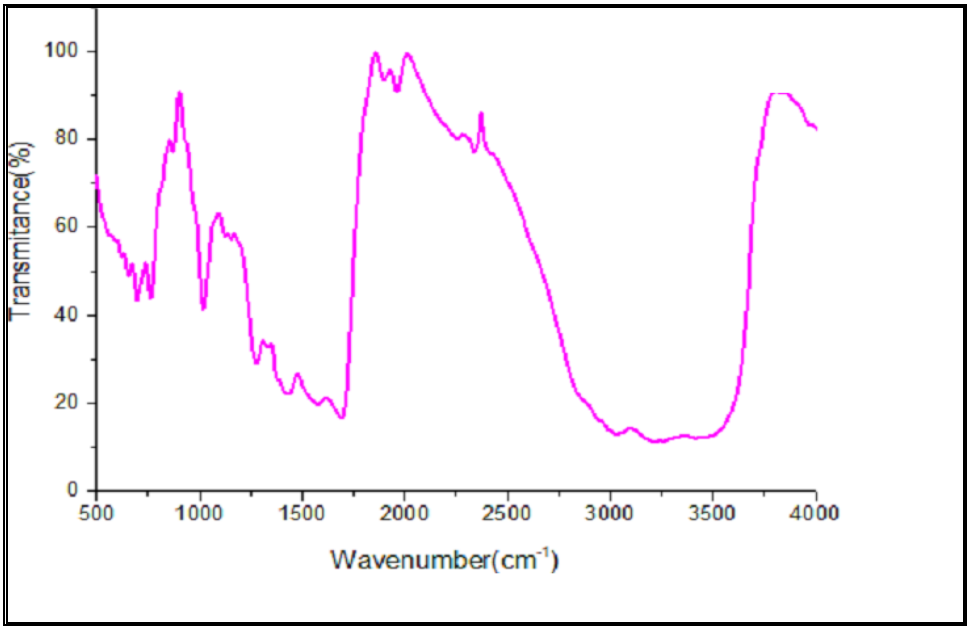
IR Interpretation: 2b



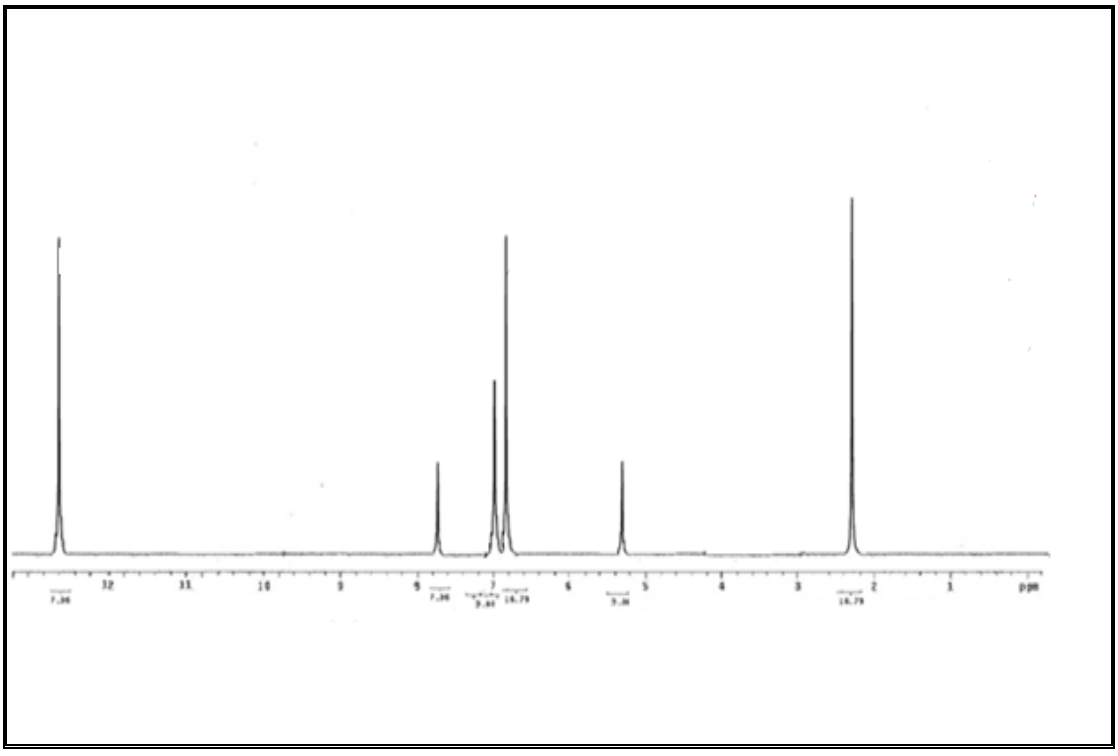
IR Interpretation: 2c



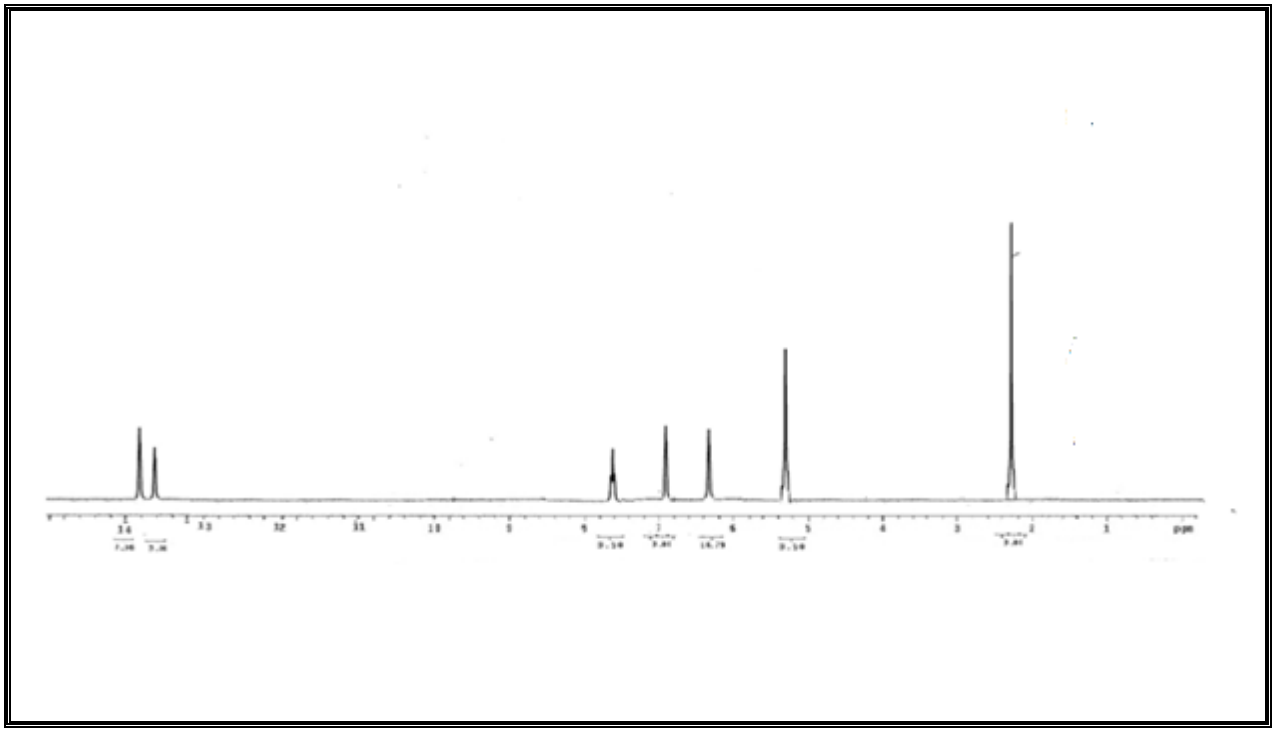
IR Interpretation: 2d



H[1]NMR Interpretation: 2a



H[1]NMR Interpretation: 2c



Antifungal activity:

Stock solution for antifungal activity: For antifungal study each compound was dissolved in DMSO at a concentration of 5mg/ml and stored in a refrigerator till further used. Antifungal activities of the compounds were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of Sabouraud dextrose agar was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121^{o}C for 15 minutes. 25 ml of molten (45^{0}C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish. For counting of spore (fungi) were suspended in normal saline to make volume up to1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened, 8mm wells were bored using a sterile cork borer. Then 0.1ml (100µl) from each stock solution of the compounds having final concentration of 5mg/ml was placed in each the well and the plates were incubated for 24 hour at 29^{o}C.Two wells in each petri dish were supplemented with DMSO and reference antifungal drug Clotrimazole (1mg/ml) dissolved in DMSO serve as negative and positive control respectively. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition. Antifungal activities of the compounds were evaluated by means of agar well diffusion assay[18,19,20.]. *Aspergillus Niger, Aspergillus Oryzae, Candida albicans, and Aspergillus flavus* were studied as follows.

Table No. 3. Antifungal study of *Pyrazole derivatives* on : *Aspergillus Niger, Aspergillus Oryzae, Candida albicans, Penicillium crysogenum and Aspergillus flavus*

Specices	**Derivatives**	**2a**	**2b**	**2c**	**2d**
Aspergillus niger	Standard antibiotic(mm)	15	15	15	15
	Control D/W	00	00	00	00
	25% (mm)	08	08	09	09
	50% (mm)	10	09	11	11
	75% (mm)	12	12	12	12
Aspergillus oryzae	Standard antibiotic(mm)	17	17	17	17
	Control D/W	00	00	00	00
	25% (mm)	08	08	09	09
	50% (mm)	12	11	11	11
	75% (mm)	14	13	16	16
Aspergillus flavus	Standard antibiotic(mm)	16	16	16	16
	Control D/W	00	00	00	00
	25% (mm)	08	10	10	09
	50% (mm)	10	12	13	11
	75% (mm)	12	15	14	14
Candida albicans	Standard antibiotic(mm)	14	14	14	14
	Control D/W	00	00	00	00
	25% (mm)	08	06	08	08
	50% (mm)	10	07	12	10
	75% (mm)	12	10	13	12

Fig.No.:2a

Fig.No.: 2b

Fig.No.: 2c

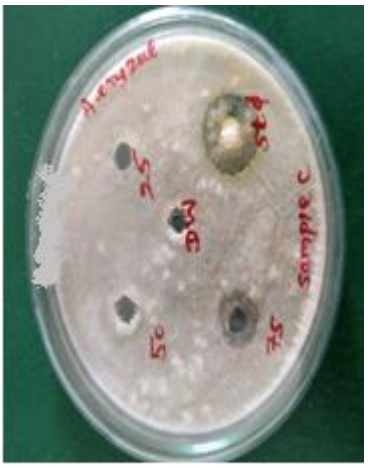


Fig.No.: 2d

Conclusion:

The pyrazole derivatives were synthesized by using chalcone was reacted with thiosemicarbazide, KOH to yield pyrazole deivatives. All the synthesized compounds were screened for antifungal. The ability of the compounds to prevent fungal was screened at 25,50,100mg/ml compared to standard.

- The compounds 2c having *o*-chloro substitutions have shown good activity
- The compounds 2a, 2b and 2d have shown significant activity.

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