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Synthesis, characterization and biological evaluation of novel pyrazole ring contain mannich derivatives

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ABSTRACT

The present article describes the synthesis of novel series of substituted phenyl hydrazono pyrazolylederivatives 4, 5, 6,7, 8, 9 and 10. All the newly synthesized target compounds Were characterized by spectral techniques like IR, NMR and Elimentalanlysis and also screened for their in antibacterial and anti fungal activity .All the tested compounds shown significant activity against gram-positive, gram-negative and fungus organisms.

Keywords: phenylhdazono, pyrazolyle, gram-positive, gram-negative, antifungal, anti bacterial activity

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INTRODUCTION

Heterocyclic compounds represents an important class of biologically active molecules specifically, those containing the pyrazolone nucleus have been shown to posses high biological activities such as tranquillizing, muscle relaxant, psycho analeptic, anticonvulsant, antihypertensive, antidepressant activities. The derivatives of pyrazolone are important class of antipyretic and analgesic Compounds[1-7]Medicinal chemists have been designed used pyrazolones extensively as scaffolds fro m which novel therapeutic agents. This heterocyclic ring system is found in a number of compounds showing analgesic morazone[8] immunosuppressant BTS-71412[9] and anti-inflammatory (aspirin–propyphenazone) activity. Numerous methods for general pyrazolone synthesis have been reported[10]Some substituted pyrazolines and their derivatives are used as antitumor[11] anti bacterial, antifungal, antiviral, anti parasitic, anti-tubercular and insecticidal agents[12-17]some of these compounds have also anti-inflammatory, anti-diabetic, and anesthetic properties[18-23] In view of this previous findings . In view of this in vitro screening of antibacterial and antifungal activity against human pathogenic bacteria, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pyogene.microbial strains, two fungis (Candida albicans and Aniger) was undertaken to study their role as microbiocidal compounds.

MATERIALS AND METHODS

General Procedures. Melting points were taken in open capillary tubes and are therefore uncorrected. Purity of the compounds was checked on silica gel G TLC plates of 2 mm thickness using n-hexane and ethylacetate as solvent system. The visualization of spot was carried out in an iodine chamber. The IR spectra of the compounds were recorded in the 4000-450 cm-1 ranges using KBr discs on FTIR IR RX1 Perkin Elmer spectropho tometers and 1H NMR were recorded on a Bruker DRX-400MHz spectrometer (CDCl3) using TMS as an internal standard. The ESI-

MS were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer having a JASCOPU-980 HPLC pump connected to it.

(A) 4-methyl- phenyl diazonium chloride

The required amount of paratoledine is dissolved in a suitable volume of water containing 3.0 equivalents of hydrochloric acid (or sulphuric acid) by the application of heat of necessary. The solution thus obtained is cooled to 0ºC when the amine hydrochloride (or sulphate) usually crystallizes. The temperature is maintained at 0-5ºC, and the aqueous solution of sodium nitrite is added portion wise till there is free nitrous acid. The solution is tested for the later with an external indicator (moist potassium iodide starch paper). An excess of acid is always maintained to stabilize the diazonium, acid is harmful; the concentration of the acid is reduced to optimum value. The similar procedure is adopted for the preparation of other substituted phenyl diazonium chlorides.

Ethyl 4,4,4-trichloro-3-oxo-2-(4-methyl phenyl hydrazono) butanoate.

A solution of sodium acetate (1.0g) in 100mL of aqueous alcohol (50%) is added to a solution of Ethyl 4,4,4-trichloroacetoacetate (0.1 mol) in 50 mL of ethanol and the mixture is added to 0ºC. to this cold mixture, the corresponding diazonium chloride is added gradually till turbidity is observed. The addition is continued till yellow crystals separated out. These crystals are filtered, washed with water and dried.

4-(4-methylphenyl hydrazono)-3-(trichloro methyl)-1H-pyrazol-5(4H)-one (2)

Mixtures of **(1)** and hydrazine hydrate and DMF (10 drops) were subjected to microwave irradiation at 150W intermittently at 30 sec intervals for 2 minutes. After complete conversion as indicated by TLC, the reaction mixture was cooled and treated with cold water. The precipitate 3-methyl 4-(4'-methyl phenyl hydrazono) pyrazoline-5-one **(2)** was filtered and recrystallized from ethanol. m.p. 180^0C, yield 87%

2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetic acid (3)

A mixture of **(2)**, 2-chloroacetic acid, anhydrous K_2CO_3 and DMF was stirred at room temperature for 8 hours. The reaction mixture was diluted with ice cold water. The separated solid was identified as 2-(5-oxo-4-(4-methyl phenyl hydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetic acid **(3)**. The ^{1}H NMR (300MHz) 3.25 (s, 3H, OCH_3), 3.62(s,2H,N CH_2CO), 10.48 (s, H, Ar-NH), 12.24 (s,1H,COOH) 6.80 -7.87 (m, 4H, for C_6H_5 phenyl group)IR (KBr) 1616 C = N 3398 **(**NH) 3424(COOH),1682 **(**C = O) 1638 **(**C = O).

2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetyl chloride (4).

To a solution of 2-(5-oxo-4-(2-(4-methyl phenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetic acid **(3)**. (900 mg) in toluene (30 mL) was added thionyl chloride (0.90 mL) at room temperatures. The resulting solution was heated to reflux for 2 h. Then, it was cooled to room temperature and the excess thionyl chloride and toluene was removed under vacuum. The residue was dissolved one time in toluene and removed again under vacuum to afford 2-(5-oxo-4-(2-(4-methyl phenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetyl chloride **(4).**The ^{1}H NMR (300MHz) 3.81(s,2H, N CH_2CO), 10.70 (s,H,Ar-NH) IR (KBr) 3380 (NH), 1696 (CO), 1617 (CN),1595(NH_{BEN}) cm^{-1};$C_{13}H_{10}Cl_4N_4O_2$; Elimental analysis Calu;C,39.42; H,2.54; N.14.15; Found; C,39.36, H,2.42;N,13.85.Yield:78%

Ethyl2-(2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetamido)propanoate (5)

A solution of acid chloride **(4)** (2.47 mmol) in dichloromethane (30 mL) were added DL-Alanine ethyl ester hydrochloride (735 mg, 2.5 mmol) and diisopropylethylamine (1.3 mL, 7.5 mmol) at 0°C. Then, the solution warmed to room temperature and it was stirred overnight. Then, it was diluted with water (50 mL) and dichloromethane (50 mL). The two layers were separated and the aqueous layer was extracted with dichloromethane (50 mL). The combined organic layer was washed with brine solution and dried over anhydrous magnesium sulfate. Filtration of the drying agent and concentration of the solvent gave the crude residue which was purified by using column chromatography to give ethyl 2-(2-(5-oxo-4-(4-methyl-phenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl) acetamido) propanoate (5) The ^{1}H NMR was recorded in DMSO-d_6 1.25-1.28 (d,3H, CH_2CH_3), 2.12-2.15(t,3H,$CHCH_3$), 3.51(s,2H, NCH_2),4.22-4.27(q,2H OCH_2) 5.18-5.25(q,1H,CH_3 CH), 10.72 (s, H, CONH), 12.58 (s, H, Ar-NH), The ^{1}H- IR (KBr) 3364,(NH), 3320 (CO-NH), 1592 (C = N) 1617(NH_{BENDI}), cyclic carbonyl 1689, 1732 (C=O)cm^{-1};$C_{18}H_{20}Cl_3N_5O_4$;Elimental analysis Calu;C,45.35; H,4.23; N.14.69; Found; C,45.29, H,4.00;N,14.60.Yield:65%

N-(1-hydrazinyl-1-oxopropan-2-yl)-2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl) -4,5-dihydro-1H-pyrazol-1-yl)acetamide (6)

A solution of **(5)** (0.01M) and hydrazine hydrate (0.015M) in ethanol 20 mL was refluxed for 5 hours. The reaction mixture was cooled and poured on to ice cold water with stirring. The separated solid was filtered, washed with water and recrystallized from ethanol to afford **(6)** their characterization data are given **.** The ^{1}H NMR (300MHz) 2.08-2.10 (d,3H, $CHCH_3$), 3.78(s,2H, NCH_2CO),4.77-4.82(q,H CH_3 CH), 9.72 (s, H, CONH), 11.18 (s, H, OH), 10.75(s, H, Ar-NH), 6.82 -7.98 (m, 5H, for C_6H_5 of phenyl group) IR (KBr) 3420,(NH_2) 3380(NH) 3198,(Ar-H) 1720(CO) 1680,(CO) 1615(CN); $C_{16}H_{18}Cl_3N_7O_3$ Elimental analysis Calu;C,41.53; H,3.92; N.21.19; Found;C,40.05, H,3.52;N,21.12.Yield:68%

N-(1-oxo-1-((Z)-2-(1-phenyl ethylidene) hydrazinyl) propan-2-yl)-2-(5-oxo-4-(4-methyl phenyl hydrazono)-3-(trichloro ethylene)-4,5-dihydro-1H-pyrazol-1-yl)acetamide (7)

To solution of **(6)** (0.01 mol) in hot methanol (25mL), acetophenone (0.01 mol) and a drop of glacial acetic acid were added. The solid that separated on refluxing for 3 hrs was filtered wash with cold methanol and recrystallized from methanol to give **(7)** m. p: 233;^{1}HNMR 1.38-1.41 (d,3H, CH_3), 3.91 (s,2H,NCH_2), 3.38(s,2H, NCH_2CO),4.34-4.38(q,CH_3CH) 9.81 (s, H, CONH), 10.74 (s, H, N -NH), 12.25 (s, H, Ar-NH), 6.48 -8.12 (m, 10H, for C_6H_5 and C_6H_4 of two phenyl groups) The ^{1}H-NMR da 3.83 (s, O–CH_3), 1.37-1.40 (d,3H, CH_3), 2.91 (s,2H,NCH_2), 3.38(s,2H, NCH_2CO),4.32-4.36(q,CH_3CH) 9.83 (s, H, CONH), 10.72 (s, H, N -NH), 12.23(s, H, Ar-NH), 6.46 -8.10 (m, 9H, for C_6H_5 and C_6H_4 of two phenyl groups) The IR (KBr) 3340,(NH)3190(Ar-H),3002(CH3), 1723(CO) 1622(CO)1604(CN)cm^{-1};$C_{24}H_{24}Cl_3N_7O_2$;; Elimental analysis Calu; C,50.63; H,4.98; N.17.42;Found; C,49.40,H,3.99;N,17.18.Yield:68%

N-(1-(4-acetyl-5-methyl-5-phenyl-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) ethyl)-2-(5-oxo-4-(4-methyl-phenyl -hydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetamide (8)

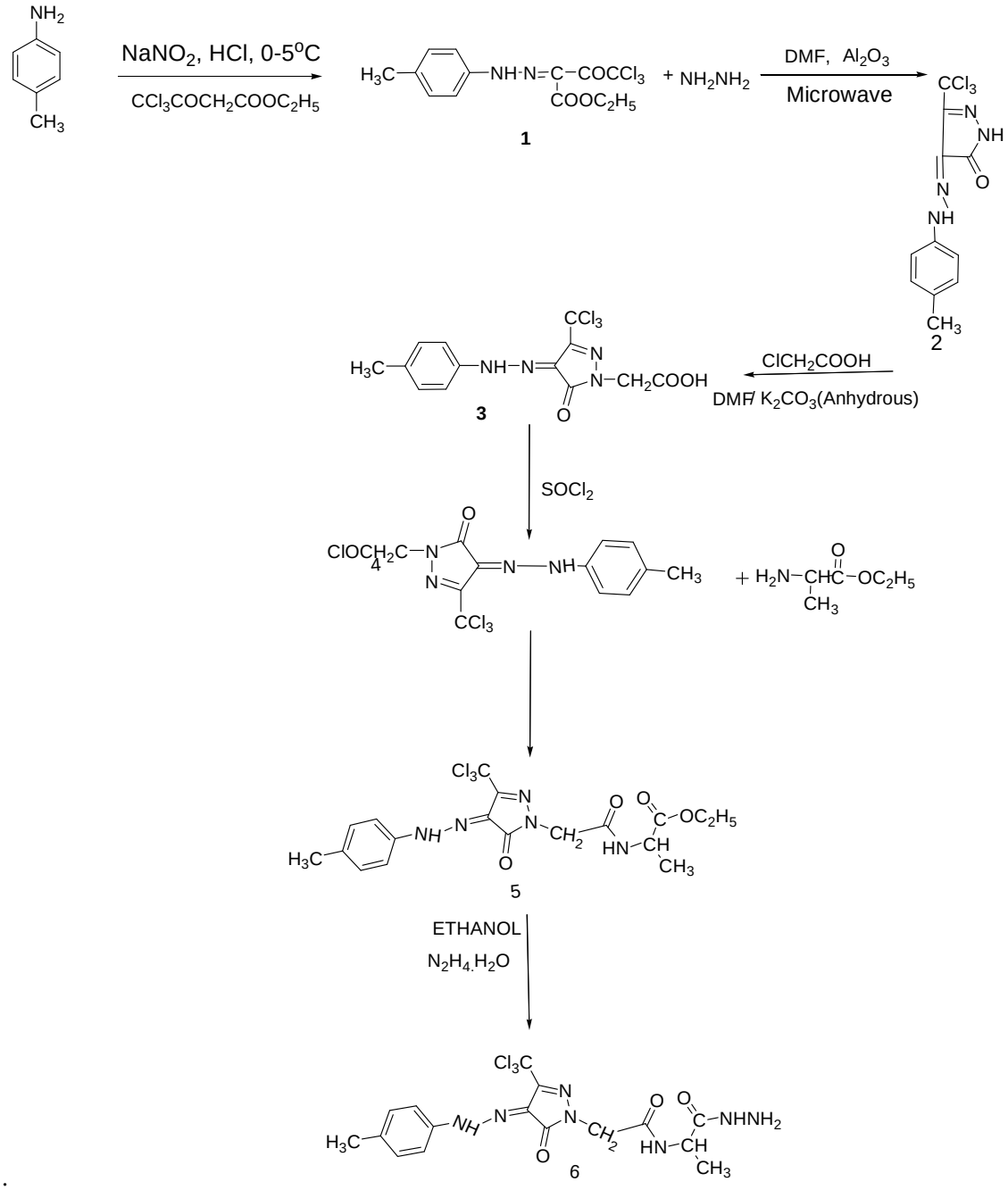
A mixture of **(7)** (0.01 mole) and excessive acetic anhydride (10 mL) was refluxed for 2 hours. The excessive acetic anhydride was distilled off and the residue was poured on to crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from aqueous methanol to furnish **(8)**, m.p.210^{0}C;The ^{1}HNMR 1.45-1.48 (d,3H,CH_3), 3.01 (s,3H,NCH_3), 3.52(s,3H, $COCH_3$), 5.52(s,2H, NCH_2CO),4.38-4.42 (q,H,CH_3CH) 10.54 (s, H, CO -NH), 12.51 (s, H, Ar-NH) The IR (KBr)3342(NH),3040,(Ar-H)1760,(CO)1680,(CO)1603(CN)and1611(CN)cm$^-$ $^1C_{26}H_{26}Cl_3N_7O_4$; Elimental analysis Calu; C,52.00; H,5.12;N.16.15;Found;C,51.00,H,4.82;N,15.98.Yield:68%

2-(5-oxo-4-(4-methyl-phenyl hydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl)acetamide (9).

A mixture of **(6)** (19.9g, 0.1 mole), KOH (5.5g, 0.1mol) ethanol (100 mL) and carbon disulphide (6.02 mL, 0.1 mol) taken in a round bottomed flask equipped with a chilled water condenser was refluxed on oil bath till the evaluation of hydrogen sulphide ceased. The excess of alcohol was removed by distillation. The reaction mixture was cooled to room temperature and the reaction mass was poured to ice cold water and neutralized with dilute hydrochloric acid. The solid precipitated was filtered, washed thoroughly with water and dried. The product was further purified by recrystallization from ethanol-dioxane mixture to give **(9)**m.p. 140^{0}C.^{1}HNMR (300MHz);1.74-1.76 (d,3H, CH_3), 3.21(S,3H,CH3), 3.95(s,2H, NCH_2CO),3.20-3.24(q,1H CH_3CH) 9.02 (s, H, CONH), 10.48 (s, 1H, thiol – thione tatomeric proton SH), 12.60 (s, H, Ar-NH), 6.72 -7.81 (m, 5H, for C_6H_5 and C_6H_5 of two phenyl groups).IR(KBr); 3392(NH),3298(NH),3142(Ar-H),1585(CN), 1780(CO), 1610 (CN),1239(CS)cm^{-1} $C_{17}H_{16}Cl_3N_7O_3S$ Elimental analysisCalu; C,40.141; H,3.99; N.19.63; Found;C, 39.52 H,3.10; N, 18.3;Yield:72%

2-(5-oxo-4-(4-methyl phenyl hydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-(5-thioxo-4-((ptolylamino) methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl)acetamide (10)

A solution of (9) (0.01 mol) in absolute ethanol and dioxane mixture (20 mL) was treated with formaldehyde (40%, 1.5mL). Later, the appropriate amine (0.01 mol) in ethanol (10 mL) was added with stirring and the reaction mixture was stirred over night at room temperature. The precipitated Mannich base was collected by filtration and dried. Recrystallization was done from ethanol-DMF mixture to give compound (10). ^{1}HNMR (300MHz) 1.82-1.85 (d,3H, CH_3),3.21(s,3H,CH3) 3.65(s,2H,NCH_2), 4.01(s,2H, NCH_2CO),2.98-3.12(q,1H CH_3CH) 10.22 (s, H, CONH), 10.72 (s, H, N -NH), 11.12 (s, H, Ar-NH), 7.02-8.14 (m, 9H, for C_6H_5 and C_6H_5 of two phenyl groups. The IR (KBr) 3360(NH),3196(Ar-H),(1730(C=O),1628(PyC=O),1260(CS),2947(CH3),1600(C=N)cm^{-1}$C_{23}H_{20}FCl_3N_8OS$ Elimental analysis Calu;C,46.12,3.28;N.18.25; Found;C,45.56H, 3.19;N, 18.15; Yield: 65%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DMF, Al2O3
Microwave
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DMF/ K2CO3(Anhydrous)
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ETHANOL
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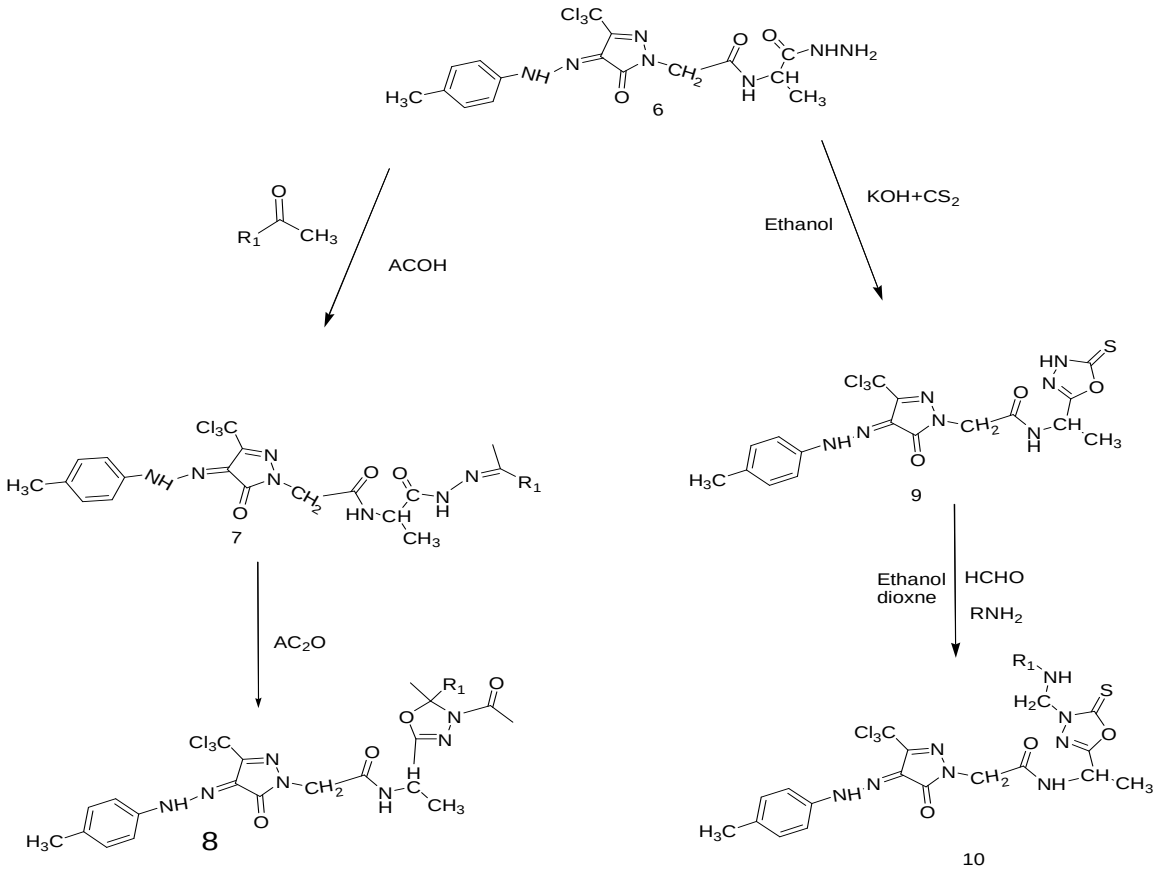
1
2
3
4
5
6



RESULTS AND DISSCUSSION

Ethyl 4,4,4-trichloro-3-oxo-2-(p-methylphenyl hydrazono) butanoate **(1)** was prepared by known procedure, A mixture of **(1)** and hydrazine hydrate and Dimethyl formamide (10 drops) was subjected to microwave irradiation at 150W intermittently at 30 seconds to get compound **(2)** 87%yield this is followed by 2-chloroacetic acid, anhydrous K_2CO_3 and DMF abtained 2-(5-oxo-4-(4-methyl-phenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)acetic acid **(3)**.The structures of these newly synthesized compound was characterized by their spectral data in yield 78% Treatment of compound 3 in thionyl chloride at room temperature. give the corresponding 2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl) acety lchlo ride **(4)** followed by the condensation of ethyle 2-amino-2-methyle acetate to give **(5**) 63% is conformed by spectral data and then presence of ethyl alcohol hydrazine hydrate treated with above resulting compound(5)to make a compound (6) Condensation of compound (6)with acetophenone compound **(7)** one the other hand compound (8)wereprepared by the reaction of aceticanhydride with (7).More over compound (6) with KOH and CS2 to gave the corresponding 2-(5-oxo-4-(4-methylphenylhydrazono)-3-(trichloromethyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(1-(5- thioxo-4,5-dihydro-1,3,4 oxadiazol-2-yl)ethyl)acetamide (9) in 63%yield. The compound (9) is involved in mannich reaction with amine and HCHO to get resulting compound (10) In vitro antimicrobial activity of tested compounds summerised in table (1) revealed the following compounds 7,8, and 10 was effective against Gram-positive and gram negative bacteria's and anti fungal activity of all compounds show hight activity

Antimicrobial activity;

All the compounds synthesized (4-10) were screened for their antibacterial and antifungal activity against human pathogenic bacteria, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pyogene. The minimum inhibition concentration (MIC) of each compound was determined using the tube dilution method. DMF was used as a blank, and Ciprofloxacin as a standard.

The compounds (**4-10**) were screened also for their antifungal activity (Table 2) against Candida albicans and Aspergillus niger using the fungicide Clotrimazole in DMF as a standard. All the compounds exhibited moderate to high antifungal activity when compared with the reference compound.

An examination of the data (Table 1) reveals that all the compounds showed antibacterial activity with an MIC ranging from 25 to 90 µg ml^{-1}. The compounds **7,8** and **10** were highly active against all the five organisms employed. The compounds **4,5,6** and **9** were moderately active against all the organisms, These results clearly indicate that the presence of a pyrazole group, acetyl group and sulphurgroups increases the antibacterial activity. Antibacterial activity (Minimum Inhibition Concentration) of compounds (**4-10)** more over all compounds exibit high antifungal activity

Table 1: antibacterial activity

Compound	Antibacterial activity (MIC, µg ml^{-1})			
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogene*
Compound-4	17	17	16	14
Compound-5	13	18	13	10
Compound-6	15	18	17	13
Compound-7	21	22	22	20
Compound-8	20	19	23	23
Compound-9	14	12	11	27
Compound-10	19	25	19	19
Ciprofloxacin	20	23	21	26

Table 2: Antifungal activity

Compound	Zone of inhibition (in mm)	
	C. albicans	*A. niger*
Compound-4	15	12
Compound-5	23	14
Compound-6	25	20
Compound-7	20	19
Compound-8	19	24
Compound-9	22	26
Compound-10	28	24
Clotrimazole(10µg/cup)	26	23

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