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SYNTHESIS, CHARACTERIZATION & ANTI-MICROBIAL ACTIVITIES OF NEW CHROMEN BASED PYRAZOLINE DERIVATIVES.

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ABSTRACT

Coumarin and pyrazolin based derivatives have reported to possess various pharmacological activities such antimicrobial, anti-inflammatory etc. It is our interest to synthesize new series of chromen based pyrazolin derivatives because of wide spectrum of activities. All the compounds synthesized using appropriate methods were purified by recrystallization and were characterized by the different methods. The melting points of the synthesized compounds were determined by using Thiel's melting point apparatus (open capillary tube method) and all the compounds gave sharp melting points and are uncorrected. Reaction was monitored by TLC using appropriate mixtures of solvents. All the final compounds were characterized & identified by IR, ^{1}H-NMR & Mass spectral data.FTIR spectra of compound showed characteristics peak of -NH at 3072.51 cm^{-1} and –COC- at 1612.02 cm^{-1}.The antimicrobial activities of the final compounds were done using broth microdilution assay. The MIC values for antibacterial studies 5d, 5g were shown good activity & 5f, 5g were shown good antifungal activity.

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INTRODUCTION

1, 2-Pyrazole is an organic compound with the formula $C_3H_3N_2$[1].It is a heterocyclic molecule characterized by 5-memberd ring of three carbon atoms and two adjacent nitrogen atoms. Pyrazole is a weak base[2].the term pyrazole was given to this class of compound by German chemist Ludwig Knorr in 1883 in a classical method developed by German chemist Hans Von Pechmann in 1898 pyrazole was synthesized from acetylene and diazomethane[3].

In medicine derivatives of pyrazole are used for analgesic, anti-inflammatory drug, antipyretics, anti-diabetic, cancer treating medicines, antifungal, antibacterial, antiviral[4].The pyrazole ring is found within a variety of pesticides as fungicides, insecticides and herbicides, including Chlorfenaprin, Fenpyroximate, Fipronil. On the other side, Coumarin is a fragrant organic chemical in the bezopyrone chemical class although it may also been as sub class of lactones it is a natural substance found in many plants and colourless [5]. crystalline substance in its standard state chemical formula $C_9H_6O_2$molar mass 146.15g mol^{-1} it is colorless to white crystals pleasant like vanilla beans odour it is soluble in either Diethyl ether chloroform, oil, pyridine, ethanol[6][7]. The name comes from a French term for the tonkabean, coumarou [8] one of the sources from which coumarin was first isolated as a natural product in 1820[9]. It has been used in perfumes since 1882. Also used in fabric conditioners. Coumarin has been used as aroma enhancers in pipe tobaccos and certain alcoholics drinks [10].Coumarin was first synthesized in 1886[11].

MATERIALS AND METHODS

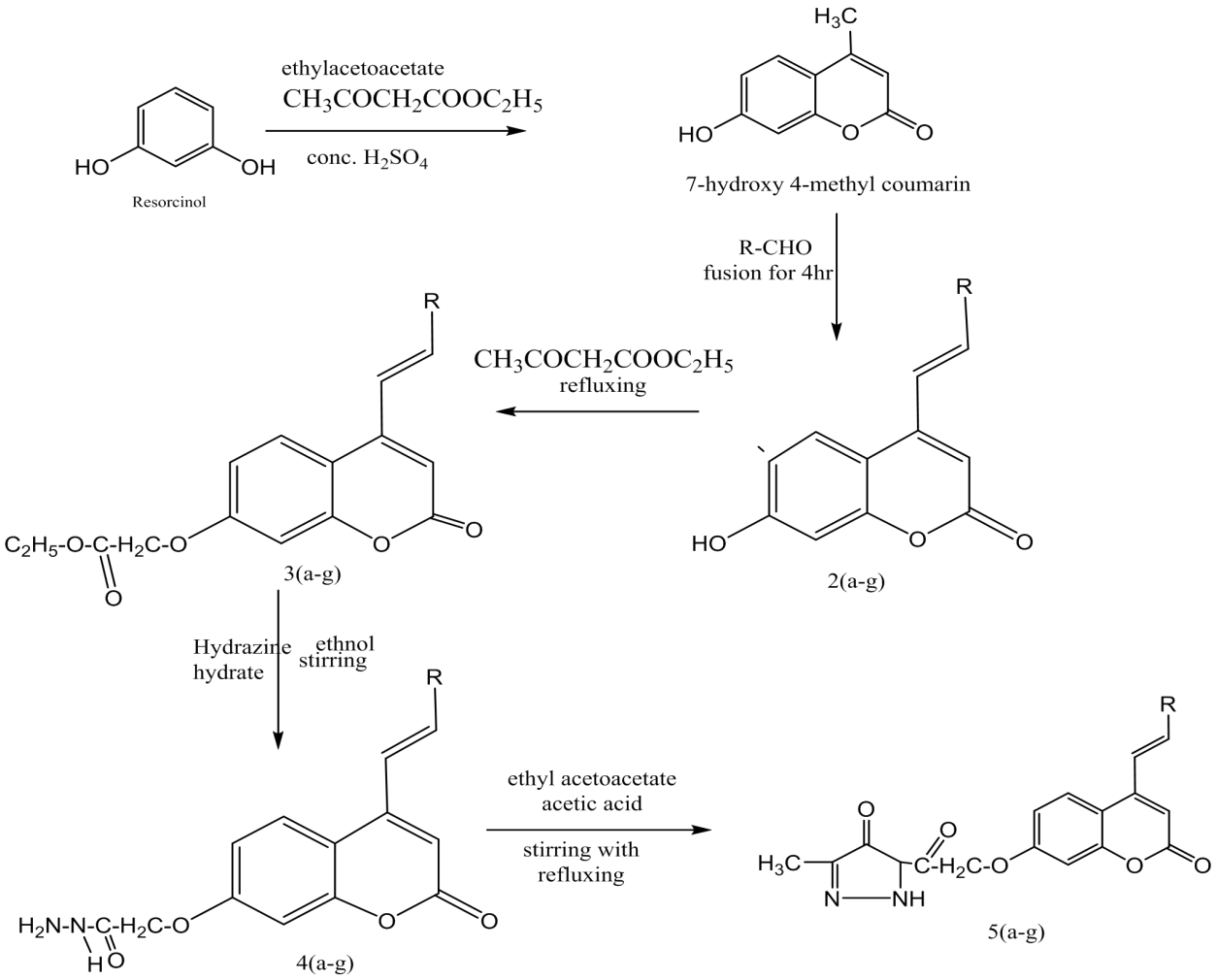
Chemicals

Chemicals used in the synthesis of the titled compounds were purchased from, Sigma-Aldrich Pvt. Ltd, HI Media laboratories, S.D. Fine Chem. Pvt. Ltd and Spectrochem Pvt. Ltd. All the solvents and chemical were purified before use by distillation/Recrystallization.

Instruments

All the melting points and boiling points of synthesized compounds were determined by capillary method in a paraffin bath/digital melting point apparatus. FT-IR spectra were recordedon Brucker alpha-T spectrophotometer by using KBr pellets and values are expressed in cm^{-1}.The ^1H NMR spectrum were recorded on Bruker 400/100 MHz instruments using DMSO-d_6/ $CDCl_3$as solvent and TMS as internal standard, chemical shifts are expressed in δ (*ppm*)as singlet (s), doublet of doublet (dd), multiplet (m). Mass spectra were recorded on Waters-Q-Tof Premier-HAB213.

SCHEME



Synthesis

Procedure for synthesis of 7-hydroxy-4-methyl coumarin from resorcinol[12]

Take 37ml of conc. H_2SO_4 in a wide beaker maintain the temperature 4-5^oC. Transfer 9.2 g of powdered resorcinol to 10.96 ml of ethylacetoacetate with constant stirring until complete solution was obtained. Add solution very slowly into conc.H_2SO_4 solution and keep stirring 30mini .pour the reaction mixture into 300ml of cold water. Stir the mixture and filter, wash again with cold water. Recrystallized from methanol.

Procedure for Synthesis of ethyl (E)-4 – (2 substituted)- 7- hydroxyl -2H- chromen- 2-one 2(a-g)[13]

0.030 moles of coumarin 0.030 moles of aromatic aldehyde dissolved in 30ml of chloroform and then catalyzed amount of piperidine 0.02 mole was added and reaction mixture was refluxed for 5 hours .Then distill of the chloroform and keep drying in china dish. Recrystallized from toluene.

Procedure for Synthesis of ethyl(E)- 2-((4-(2- substituted)- 2-oxo- 2H- chromen -7- yl) oxy) acetate. 3(a-g)[13]

Take 0.01mole of p-acetamido phenol 25ml of dry acetone and 0.01mole of ethyl chloro acetate 2 g of potassium carbonate reflux it for 3-4 days then pour it in to crushed ice precipitate came then filter the solution. Recrystallized from ethanol.

Procedure for Ethyl (E) 2- ((2-oxo-4-(substituted)-2H- chromen-7-yl) acetohydrazide. 4(a-g)[13]

0.0049 mole of ester was stirred with hydrazine hydrate (0.2ml) in ethanol over night. After the reaction has finished the precipitate was filtered out and Recrystallized from ethanol.

Procedure forEthyl(E)-2- ((4-2(substituted) -2-oxo-2H-chromen -7-yl) oxy)-1- oxoethan-1-ide 3-methyl-1,5- dihydro -4H-pyrazole-4-one. 5(a-g)[14]

0.011 mole of hydrazide and 0.01 mole of ethyl acetoacetate in 20ml of acetic acid stirring with refluxing for 24hours. The reaction mixture was evaporated till dryness, the product was collected and washed with ethanol and dried. Recrystallized from ethanol.

Microbiological Screening

Evaluation of antibacterial activity

The Minimum Inhibitory Concentration (MIC) determination of the tested compounds were investigated in side-by-side comparison with ciprofloxacin against Gram-positive (Staphylococcus aureus,BacillusSubtilis) and Gram-negative bacteria (Klebsiellapneumoniae, Escherichia coli) by broth micro dilution method.

MATERIALS AND METHODS

1. Mueller-Hinton agar
2. McFarland turbidity standards
3. Scrupulously clean, acid-washed borosilicate glass tubes
4. Micropipette
5. Nutrient agar

Preparation of media

Sterilization of media and glassware

The media used in the present study, Mueller-Hinton agar and nutrient agar were sterilized in conical flasks of suitable capacity by autoclaving at 15lb pressure for about 20 minutes. The test tubes and pipettes were sterilized in hot air oven at 160^0C for 1h.

Preparation of solution of test compounds

Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (1mg) were dissolved in chloroform ($CHCl_3$, 1ml). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml.

Preparation of the Inoculums

The organisms were sub-cultured on to nutrient agar and incubated overnight at 35^0C. The tubes that contain 2ml of Mueller-Hinton agar inoculated with five or more colonies from the agar plate and turbidity was adjusted to match a 1 McFarland standard (10^5cfu/ml) and incubated at 37^0 C for 18h. The MIC was the lowest concentration of the tested compound that yields no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with $CHCl_3$ at the same dilutions as used in the experiments and $CHCl_3$ had no effect on the microorganisms in the concentrations studied.

Evaluation of antifungal activity

The Minimum Inhibitory Concentration (MIC) determination of the tested compounds were investigated in side-by-side comparison with fluconazole against a panel of standard strains of Candida albicans, Aspergillusniger and Aspergillusflavusby broth micro dilution method.

MATERIALS AND METHODS

1. Sabouraud Dextrose Agar (SDA)
2. McFarland turbidity standards
3. Scrupulously clean, acid-washed borosilicate glass tubes
4. Micropipette
5. Nutrient agar

Preparation of media:

Sterilization of media and glassware

The media used in the present study, Sabouraud Dextrose agar and nutrient agar were sterilized in conical flasks of suitable capacity by autoclaving at 15lb pressure for about 20 minutes. The test tubes and pipettes were sterilized in hot air oven at 160^0C for 1 h.

Preparation of solution of test compounds

Serial dilutions of the test compounds and reference drugs were prepared in Sabouraud Dextrose agar. Drugs (1mg) were dissolved in chloroform ($CHCl_3$, 1ml). Further progressive dilutions with melted Sabouraud Dextrose agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml.

Preparation of the Inoculums

The organisms were sub-cultured on to nutrient agar and incubated overnight at 35^0C. The tubes that contain 2ml of Sabouraud Dextrose agar inoculated with five or more colonies from the agar plate and turbidity was adjusted to match a 1 McFarland standard (10^5cfu/ml) and incubated at 37^0 C for 18h. The MIC was the lowest concentration of the tested compound that yields no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with $CHCl_3$ at the same dilutions as used in the experiments and $CHCl_3$ had no effect on the microorganisms in the concentrations studied.

RESULTS

Ethyl (E) -2- ((4-2(benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one.(5a) Yield:-75%, M.P140-143 FTIR (KBr cm^{-1}) 3072.51,3072.51(N-Hstr), 2932.25 2857.57(CH)1713.88(C=O)1612.60(C=C)1433.60(C-Hbend)1277.27(C-N);^{1}HNMR(δppm)10.0(1H,NH)6.22(1H,CH)4.12(1H,CH)

Ethyl (E) -2- ((4-2(4-methyl benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5b) Yield:- 68%, , M.P 145-49, IR (KBr cm^{-1}) 3153.14,3405.80(N-Hstr), 2922.00,2854.74(C-Hstr), 1899.54(C=O), 1723.42(C=O), 1619.48(C=C), 1433.27(C-Hbend), ^{1}H-NMR (δ ppm) 10.8(1H,NH)7.73(CH_2)6.25(CH)3.59(CH_2)2.41(CH_3).

Ethyl (E) -2- ((4-2(4-methoxy benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5c) Yield:-78%, M.P 138-41,FTIR (KBr cm^{-1}) 3166.09(N-Hstr), 3067.66,2923.21(C-Hstr), 1723.75(C=O), 1613.76(C=C), 1431.04(C-H bend), ^{1}H-NMR(δppm)10.8(1H,NH), 7.79(2H,CH_2), 7.04(2H,CH_2), 3.36(1H,CH).

Ethyl (E) -2- ((4-2(4-fluro benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5d) Yield:-75%, M.P 140-44,FTIR (KBr cm^{-1}) 3157.23(N-Hstr),2922.34,2854.43(C-Hstr), 1720.37(C=O), 1434.11(C-H bend),1389.78(C-F).

Ethyl (E) -2- ((4-2(2-chloro benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5e) Yield:-74%, M.P 148-52, FTIR (KBrcm^{-1}) 3072.51,3072.51(N-Hstr),2932.25,2857.57(C-Hstr),1713.88(C=O),1612.60(C=C),1433.60(C-Hbend), 617.06,750.59(C-Cl), ^{1}H-NMR(δppm) 10.3(1H,NH), 3.31(1H,CH), 7.83(1H,CH), 7.72(1H,CH).

Ethyl (E) -2- ((4-2(2-chloro benzaldehyde)–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5f) Yield:-80%, M.P 138-42, FTIR (KBr cm^{-1}) 3159.14,3492.67(N-Hstr), 3102.32(C-Hstr), 1669.62(C=O), 1603.52(C=C), 1449.33(C-H bend), 637.82(C-Br)

Ethyl (E) -2- ((4-2–2-oxo-2H-chromen-7-yl)oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one. (5g) Yield:-80%, M.P 138-42, FTIR (KBr cm^{-1}) 3153.14,3405.80(N-Hstr), 2922.00,2854.74(C-Hstr), 1899.54(C=O), 1723.42(C=O), 1619.48(C=C), 1433.27(C-Hbend), 3542.23(O-H) ^{1}H-NMR(δppm) 10.01(1H,NH), 7.72(CH_2), 6.24(CH),2.43(CH_3),2.16(CH_3).

Antibacterial Activity:

All the newly synthesized compounds were screened for the antibacterialby broth micro dilution assay method. Ciprofloxacinwas used as standard drugs and activitiesof all newly synthesized compounds was measured against it & are depicted in Table No. 8.

Table No. 8: MIC (µg/ml) of synthesized compounds 5(a-g).

Compounds	R	Gram +ve		Gram –ve	
		Staphylococcus Aureus	Bacillus Subtilis	Klebsiella Pneumoniae	Escherichia Coli
5a	H	125	16	62.5	31.25
5b	4-CH_3	31.25	62.5	125	500
5c	4-OCH_3	25	31.25	31.25	2.5
5d	4-NO_2	2	4	8	4
5e	4-Br	4	6	8	2
5f	4-Cl	2	8	4	2
5g	4-F	2	6	10	2
Ciprofloxacin		**2**	**2**	**2**	**2**

Antifungal Activity:

All the newly synthesized compounds were screened for the antifungal activities by broth micro dilution assay method. Fluconazole was used as standard drug and activity of all newly synthesized compounds was measured against it & are depicted in Table No. 9.

Table No. 9: MIC (µg/ml) of synthesized compounds 5(a-g).

Compounds	R	Candida Species	Aspergillus Species	
		Candida Albicans	Aspergillus Niger	AspergillusFlavus
5a	H	16	62.5	25
5b	4-CH_3	25	62.5	16
5c	4-OCH_3	10	16	125
5d	4-NO_2	6	8	6
5e	4-Br	4	2	8
5f	4-Cl	2	8	4
5g	4-F	2	4	2
Fluconazole		**1**	**1**	**1**

DISCUSSION

The pyrazole were synthesized by cyclisation of different hydrazides. Hydrazides were made to react with ethyl aceto acetate. Acetic acid was used as solvent. IR spectra of compound showed characteristics peak of -NH at 3072.51 cm^{-1} and –COC- at 1612.02 cm^{-1} which confirms the structure of compounds. IR spectra of compound showed characteristic absorption peak of C-N near 1277.27cm^{-1} , C-H (Aromatic) near 2857.57 cm^{-1} , C-H (Aliphatic) near 2952.32 cm^{-1}, -NHnear 3072.51cm^{-1}, C-C (Aromatic) near 1433.31 cm^{-1}, C-O-C near 1052.14 cm^{-1}which confirms the structure of the compounds.Minimum Inhibitory Concentration (MIC) for antibacterial and antifungal activity was determined by broth micro dilution method usingMuller-Hinton and Sabouraud Dextrose agar medium as media against *Staphylococcus aureus, Bacillus subtillis, Escherichia colli and Klebissella Pneumoniae* and compared with Ciprofloxacin and *against Candida albicans, Aspergill usniger and As per gillusflavus* and compared with Fluconazole as standard respectively. MIC showed that many of the compounds were active against the microorganism in very minimal concentrations. Among the series of compounds 5d, 5g showed good antibacterial activity and 5e and 5f showed moderate antibacterial activity and series of compounds 5f and 5gshowed excellent antifungal activity. The compounds5f and 5g were found active against Gram positive bacteria i.e. *Staphylococcus aureus* at concentration of (2µg/ml). The compounds5e,5f and 5g were found active against Gram negative bacteria i.e. *Escherichia Coli* at concentration of (2µg/ml). The compound 5d, 5f and 5gshowed excellent antifungal activity against *Candida Albicans* and *As per gillus Niger* at concentration of(4µg/ml).

CONCLUSION

In view of all the mentioned observations & results, it is concluded that the out of 7 compounds synthesized, compound 5f has shown excellent antimicrobial activity due to the presence of electron withdrawing group. All the compounds are of good purity & standards. Further research can be explored with the possible changes to the existing moiety.

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Authors' agreements

Authors hereby declare that there is no conflict of interest for the publication.

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