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Synthesis Charecterization and Antimicrobial Activity of New Series of Chromone Based Pyrazoline Derivatives

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ABSTRACT:

Coumarin and pyrazolin have reported to possess various pharmacological activities along with wide range of anti-microbial activity. It is our interest to synthesize some new chromone based pyrazolin derivatives and to evaluate their anti-microbial activity.

Interpretation:All the compounds synthesized were purified by recrystallization and were characterized by the following methods:

Melting point determination: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.

Thin layer chromatography:Purity of the compounds was ascertained by TLC using appropriate mixtures of the following solvents *n*-hexane: ethyl acetate(6:4)

Infrared spectroscopy:The IR spectral analysis was carried out using FT-IR (SHIMADZU 8400S/Brucker).

Nuclear Magnetic Resonance spectroscopy:^{1}H-NMR spectral analysis of compounds was carried out on AMX-400 NMR spectrometer (Bruker) at Punjab University.

Anti-microbial activity: The antibacterial activity of synthetic compounds was determined against a panel of standard strains of the Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Klebsiellapneumoniae, Escherichia coli*) by broth micro dilution method.The antifungal activity of synthetic compounds was determined against a panel of standard strains of Candida*albicans,* and *Aspergillusniger, Aspergillusflavus.*

Keywords: Pyrazole, Chromone, anti-microbial activity

Introduction:

Heterocyclic chemistry is the branch of chemistry dealing exclusively with synthesis, properties and applications of heterocyclic's especially vital to drug design.Incorporation of oxygen, nitrogen, sulfur, or an atom of a related element into an organic ring structure in place of a carbon atom gives rise to a heterocyclic compound. Since the heterocyclic atom must form more than one bond in order to be incorporated into a ring structure, halogens do not form heterocyclic compounds although they may be substituent on a heterocyclic ring structure. Heterocyclic compounds like polycyclic ring compounds, are usually known by non-systematic names.[1] In the family of heterocyclic compounds nitrogen containing heterocyclic are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes.[2]

Azoles are five member heterocyclic compounds containing in their rings one or more hetero atoms, at least one of which is nitrogen. Standard drugs used in some of the medicinally important derivatives containing azoles are Novalgin, Aminopyrine etc. which possess NSAID properties. Apart from this, Benzimidazoles, Triazoles possess different biological activities like antimalarial, hypertensive and antifungal.[3]

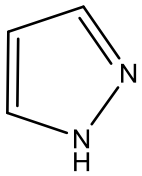
Discovery of antimicrobial agents was the most brilliant achievement of modern medicine in the 20th century. In the "golden age of antibiotics" from 1940s to 1970s, antimicrobial agents have significantly increased human life expectancy by curing previously fatal infectious diseases. At the end of 1960s, some authorities impetuously declared that infectious diseases would have been the history of human life. Currently, however, half a century after the introduction of "miracle drugs", scientific community and public fear the re-emergenceof infectious diseases caused by antibiotic-resistant bacteria. Most cardinal infectious diseases which account for more than 85 % of the mortality from infection worldwide such as acute respiratory infections, diarrheal diseases, AIDS, malaria, and tuberculosis have serious problems in the treatment due to widespread emergence of antimicrobial resistance. For example, treatment of acute respiratory infections is complicated by the emergence of pneumococcal resistance to penicillin, macrolides, and

fluoroquinolones. Antimicrobial resistance among major human pathogens including bacteria, virus, fungi, and mycobacteria is obviously one of the most serious threat to public health globally in the 21st century.

Antimicrobial resistance results in increased morbidity and mortality from treatment failures and increased health care costs. It is estimated that USD 30 billion is spent on the cumulative effects of antimicrobial resistance each year including multiple drug regimens, extra hospital days, additional medical care and lost productivity. And a recent study showed that multidrug-resistance was associated with increasing incidence of invasive pneumococcal diseases in children younger than 5 years of age. It means that antibiotic resistance directly contributes to increasing incidence of invasive diseases.[4-7] Therefore, there is an urge to synthesize more potent derivatives containing these atoms and this research is an attempt to synthesize better, effective Oxygen & Nitrogen heterocyclic derivatives.

1a. PYRAZOLE

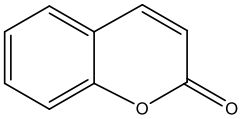
Pyrazole is an organic compound with the formula $C_3H_3N_2$[8].it is a heterocyclic characterized by a 5-memberd ring of three carbon atoms and two adjacent nitrogen atoms. Pyrazole is a weak base.[9] the term pyrazole was given to this class of compound by German chemist Ludwig Knorr in 1883 in a classical method developed by German chemist Hans Von Pechmann in 1898 pyrazole was synthesized from acetylene and diazomethane.[10]In medicine derivatives of pyrazole are used for analgesic, anti-inflammatory drug, antipyretics, anti diabetic, cancer treating medicines, antifungal, antibacterial, antiviral.[11]



PYRAZOLE

The pyrazole ring is found within a variety of pesticides as fungicides, insecticides and herbicides, including Chlorfenaprin, Fenpyroximate, Fipronil. Imidazole is an analogof pyrazolewith two non-adjacent nitrogen atoms.[12]

COUMARIN (Chromone)



COUMARIN

It is a fragrant organic chemical in the bezopyrone chemical class although it may also been as sub class of lactones[9] it is a natural substance found in many plants and clourless[13] 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.[14 15] The name comes from a French term for the tonkabean, coumarou[16] one of the sources from which coumarin was first isolated as a natural product in 1820.[17]It has been used in perfumes since 1882. Also used in fabric conditioners. Coumarin has been used as an aroma enhancers in pipe tobaccos and certain alcoholics drinks.[18]Coumarin was first synthesized in 1886.[19]

Objectives:

Nitrogen containing heterocyclic compounds constitutes an important class of compounds in medicinal chemistry. There has been considerable interest in the development of synthetic methods for the production of coumarin and pyrazoline. This seems to be because they represent very active classes of compounds possessing a wide spectrum of biological activities.

The main objectives of present project can be summarized as follows:

1) To synthesize some new chromone based pyrazoline derivatives.
2) To purify the compounds and characterize the structure of the newly synthesized compounds by different analytical techniques such as IR, NMR and Mass spectral data.
3) To evaluate the characterized compounds for *in-vitro* anti-microbial activities.

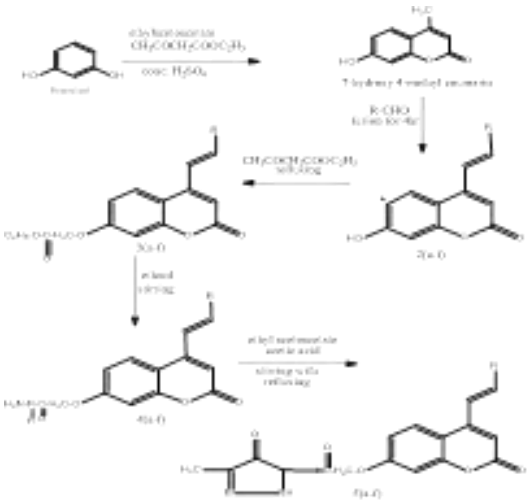
4.1. Materials and Methods

4.2. **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 SpectrochemPvt. Ltd. All the solvents and chemical were purified before use by distillation/Recrystallization.

4.3. 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 recorded onBrucker alpha-T spectrophotometer by using KBr pellets and values are expressed in cm^{-1}.

The ^1H NMR and spectrum where recorded on Bruker 400/100 MHz instruments using DMSO-d_6/ CDCl3as 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



4.4 SYNTHESIS

5. Synthesis of 7-hydroxy 4-methyl coumarin from resorcinol[51]

Take 37ml of conc. H_2SO_4 in a wide beaker maintain the temperature 4-5°C. Transfer 9.2 g of powdered resorcinol to 10.96 ml of ethylacetoacetate with constant stirring until complete solution is 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.

2. Synthesis of ethyl (E)-4 – (2 substituted)- 7- hydroxyl -2H- chromen- 2-one 2(a-g)[52]

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 is added and reaction mixture is refluxed for 5 hours .Then distill of the chloroform and keep drying in china dish. Recrystallized from toluene.

3. Synthesis of ethyl(E)- 2-((4-(2- substituted)- 2-oxo- 2H- chromen -7- yl) oxy) acetate. 3(a-g)[52]

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.

4. Ethyl (E) 2- ((2-oxo-4-(substituted)-2H- chromen-7-yl) acetohydrazide. 4(a-g)[52]

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.

5. Ethyl(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)[53]

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.

4.4 MICROBIOLOGICAL SCREENING:

4) <u>Antimicrobial activity:</u>

a) <u>Antibacterial Activity</u>:

Evaluation of antibacterial activity:

The Minimum Inhibitory Concentration (MIC) determination of the tested compounds was investigated in side-by-side comparison with ciprofloxacin against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria *(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 1 h.

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 Muller-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.

b) Antifungal activity: The Minimum Inhibitory Concentration (MIC) determination of the tested compounds were investigated in side-by-side comparison with Fluconozole against Candida albicans, Aspergillusniger by broth micro dilution method.

Materials and methods:

1. Sabouraud-dextrose 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, 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.

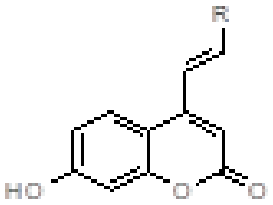
Preparation of solution of test compounds: Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (1mg) were dissolved in chloroform ($CHCl_3$, 1ml). Further progressive dilutions with melted Sabouraud-dextrose agar were performed to obtain the required 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.

5. RESULTS AND DISCUSSION

Table No. 1. Physico-chemical properties of Ethyl (E) -2- ((2-oxo-4 –substituted – 7-hydroxy-2H- chromen-2-one 2(a-g)

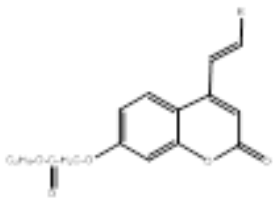
Sl. No.	Compound Code	R	Nature of compound	Molecular Formula	Mol. Wt.	MP(^{0}C)	RT(hr)	% yield	$R_f$value
1	2a	…….	White crystalline	$C_{10}H_8O_3$	176	162-65	4	75	0.5
2	2b	C_6H_5	White crystalline	$C_{17}H_{12}O_3$	264	170-73	3.5	80	0.52
3	2c	4-CH_3-C_6H_5	White crystalline	$C_{18}H_{15}O_4$	295	168-70	4.5	82	0.55
4	2d	4-OCH_3-C_6H_5	White crystalline	$C_{18}H_{15}O_3$	279	160-64	5	78	0.53
5	2e	2-Cl-C_6H_5	White crystalline	$C_{17}H_{11}O_3Cl$	280	165-68	3.5	85	0.56
6	2f	2-Br-C_6H_5	White crystalline	$C_{17}H_{11}O_3Br$	279	158-60	5.5	79	0.52
7	2g	4-F-C_6H_5	White crystalline	$C_{17}H_{11}O_3F$	272	150-55	4	85	0.55



Recrystallization Solvent: Ethanol.

Table No2. Physico-chemical properties of ethyl (E)-2- -4- (2 substituted) -2-oxo-2H chromen-7-yl) oxy) acetate. 3(a-g)

Sl. No.	Compound Code	R	Nature of compound	Molecular Formula	Mol. Wt.	MP(^{0}C)	RT(hr)	% yield	$R_f$value
1	3a	…….	White powder	$C_{14}H_{14}O_5$	262	95-97	0.58	36	80
2	3b	C_6H_5	White powder	$C_{21}H_{18}O_5$	350	92-95	0.55	42	82
3	3c	4-CH_3-C_6H_5	White powder	$C_{22}H_{19}O_6$	362	97-100	0.62	49	87
4	3d	4-$OCH_3C_6H_5$	White powder	$C_{22}H_{19}O_5$	364	100-05	0.67	49	67
5	3e	2-Cl-C_6H_5	White powder	$C_{21}H_{17}O_5Cl$	384	98-100	0.7	52	83
6	3f	2-Br-C_6H_5	White powder	$C_{21}H_{17}O_5Br$	366	102-05	0.66	49	66
7	3g	4-F-C_6H_5	White powder	$C_{21}H_{17}O_5F$	358	105-10	0.68	54	72



Recrystallization Solvent: Ethanol

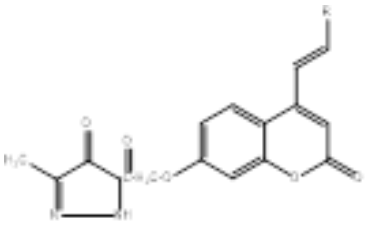
Table No. 3. Physico-chemical properties of Ethyl(E)-2-((2-oxo-4-(substituted) -2H-chromen -7-yl) acetohydrazide.4(a-g)

Sl.No.	Compound Code	R	Nature of compound	Molecular Formula	Mol. Wt.	MP(^{0}C)	RT(hr)	% yield	$R_f$value
1	4a	…….	White powder	$C_{12}H_{12}O_4N_2$	234	198-200	O.71	77	12
2	4b	C_6H_5	White powder	$C_{19}H_{16}O_4N_2$	336	200-205	0.69	73	12
3	4c	4-CH_3-C_6H_5	White powder	$C_{20}H_{19}O_4\ N_2$	348	205-08	0.75	80	14
4	4d	4-$OCH_3C_6H_5$	White powder	$C_{20}H_{19}O_5N_2$	350	204-07	0.76	71	13
5	4e	2-Cl-C_6H_5	White powder	$C_{19}H_{16}O_4ClN_2$	370	215-18	0.79	80	15
6	4f	2-Br-C_6H_5	White powder	$C_{19}H_{16}O_4BrN_2$	352	210-15	0.76	75	12
7	4g	4-F-C_6H_5	White powder	$C_{19}H_{16}O_4FN_2$	331	220-23	0.75	84	14

Recrystallization Solvent: Ethanol

Table No. 4. Physico-chemical properties of (E)-2-((4-2(substituted)-2-oxo-2H-chromen-7-yl)oxy)-1- oxoethan-1 –ide-3-methyl-1,5-dihydro-4H-pyrazol-4-one.5(a-g)

SL.NO	Compounds	R	Nature of compound	Mol.F	Mol,wt	Melting Point	R_f value	% yield	RT(hr)
1	$_{5a}$	…….	White crystalline	$C_{24}H_{22}O_5N_2$	404	135-38	0.63	70	12
2	5b	C_6H_5	White crystalline	$C_{19}H_{19}O_5N_2$	314	140-43	0.67	75	13
3	5c	4-CH_3-C_6H_5	White crystalline	$C_{19}H_{19}O_6N_2$	357	145-49	0.65	68	14
4	5d	4-$OCH_3C_6H_5$	White crystalline	$C_{18}H_{16}O_5N_2F$	335	138-41	0.64	78	12
5	5e	2-Cl-C_6H_5	White crystalline	$C_{18}H_{16}O_5N_2Cl$	343	148-52	0.6	74	14
6	5f	2-Br-C_6H_5	White crystalline	$C_{18}H_{16}O_5N_2Br$	361	138-42	0.59	80	15
7	5g	4-F-C_6H_5	White crystalline	$C_{18}H_{16}O_5N_2$	326	140-44	0.64	75	13



Recrystallization solvent: ethanol

Table No. 5. Spectral data of 7-hydroxy 4- methyl coumarin

Comp. No.	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
1	3444.83(OH str), 2821.76(C-H str), 2233.80(C=Ostr), 1450.59(C-H str)	-------	-------

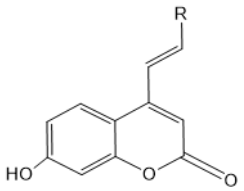
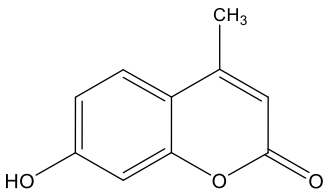


Table No.6.Spectaral data of Ethyl (E)-2-((2-oxo-4-(substituted) -2H- chromen- 7-yl) oxy) acetate.2(a-g)



Comp. No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
2e	2-Br C_6H_5	3156.78(OHstr), 2932.89(C-Hstr),1798.98(C=Ostr),1681.10(C=Cstr),637.82(C-Br str)		
2f	2-Cl C_6H_5	3156.78(OHstr), 2932.89(C-Hstr),1798.98(C=Ostr),1681.10(C=Cstr),747.71(C-Clstr)		

Table No.7.Spectral data of Ethyl(E)-2-((4-(2-substituted)2-oxo-2H- chromen-7-yl) oxy) acetate.3(a-g)

Comp. No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
3a	C_6H_5	3063.92(C-Hstr)aromatic, 2979.80(C-Hstr)alkane 1741.19(C=Ostr)1614.56 (C=Cstr)1445.17,(C-H str)	--------	--------
3b	4-CH_3 C_6H_5	3487.12(C-H)aromatic, 2979.80(C-H)alkane1741.19(C=O)1614.56(C=C)1445.17, (CH_3)	--------	--------
3c	4-OCH_3 C_6H_5	3127.49(C-H)aromatic, 2979.80(C-H)alkane1742.19(C=O)1614.56(C=C)1445.75(CH_3)	--------	--------
3d	4-F C_6H_5	3157.48(C-H)aromatic, 2979.80(C-H)alkane1742.19(C=O)1614.56(C=C)1448.02(CH_3)1120.79(C-F)	--------	--------

3e	2-Cl C_6H_5	3157.89(C-H)aromatic, 2921.61(C-H)alkane1676.90(C=O)1600.41(C=C)1450.47 (CH_3)749.35(C-Cl)	--------	--------
3f	2-Br C_6H_5	3129.31(C-H)aromatic, 2982.60(C-H)alkane1613.59(C=O)1600.41(C=C)1445.25(CH_3)621.46(C-Br)	--------	--------
3g	------	3065.05(C-H)aromatic, 2981.82(C-H)alkane1614.33(C=O)1737.17(C=C)1445.47(CH_3)	--------	--------

Table No.8. Spectral data of Ethyl (E)-2-((2-oxo-4-(substituted)-2H-chromen-7-yl) acetohydrazide.4(a-g)

Comp. No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
4a	-C_6H_5	3328.91(N-H), 3267.75(NH_2),1726.25(C=O),1433.60(CH_2),1675.27(C=C), 1726.25(C=O)	----------	--------
4b	4-CH_3-C_6H_5	3328.83(N-H), 3267.62(NH_2),1726.93(C=O),1435.46(CH_2),1676.26(C=C), 1383.99(CH_3)	----------	--------
4c	4-OCH_3 C_6H_5	3329.81(N-H), 3268.32(NH_2),1726.29(C=O),1434.34(CH_2),1675.94(C=C), 1385.18(CH_3)		348
4d	4-F C_6H_5	3331.66(N-H), 3273.04(NH_2),1725.31(C=O),1436.81(CH_2),1678.02(C=C), 1385.29(C-F)		350
4e	2-Cl C_6H_5	3443.61(N-H), 3112.06(NH_2),1672.04(C=O),1450.37(CH_2),1607.16(C=C), 748.08(C-Cl).		370
4f	2-Br C_6H_5	3269.83(N-H), 3082.86(NH_2),1680.80(C=O),1437.17(CH_2),1610.15(C=C), 673.16(C-Br)		352
4g	------	3331.52(N-H), 3269.26(NH_2),1726.78(C=O),1435.32(CH_2),1676.75(C=C), 1386.66(CH_3)		331

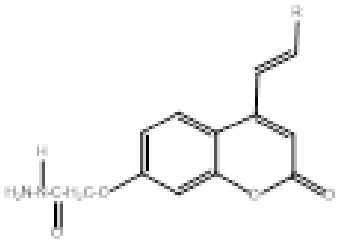
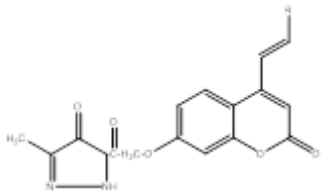


Table No.9. Spectral data of Ethyl(E)-2-((4-2(substituted)-2-oxo-2H-chromen -7-yl) oxy)-1-oxoetan-1-ide 3-methyl-1,5-dihydro-4H-pyrazole-4-one.5(a-f)



Comp. No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
5a	C_6H_5	3072.51,3072.51(N-H),2932.25,2857.57(C-H)1713.88(C=O)1612.60(C=C)1433.60(CH_3)	----------	404
5b	4-CH_3 C_6H_5	3153.14,3405.80(N-H), 2922.00,2854.74(C-H), 1899.54(C=O), 1723.42(C=O), 1619.48(C=C), 1433.27(CH_3)	----------	314
5c	4-OCH_3 C_6H_5	3166.09(N-H), 3067.66,2923.21(C-H), 1723.75(C=O), 1613.76(C=C), 1431.04(CH_3)	----------	--------
5d	4-F C_6H_5	3157.23(N-H),2922.34,2854.43(C-H), 1720.37(C=O), 1434.11(CH_3),1389.78(C-F)	----------	335
5e	2-Cl C_6H_5	3072.51,3072.51(N-H),2932.25,2857.57(C-H)1713.88(C=O)1612.60(C=C)1433.60(CH_3)617.06,750.59(C-Cl)	----------	--------
5f	2-Br C_6H_5	3159.14,3492.67(N-H), 3102.32(C-H), 1669.62(C=O), 1603.52(C=C), 1449.33(CH_3), 637.82(C-Br)	----------	--------
5g	------	3153.14,3405.80(N-H), 2922.00,2854.74(C-H), 1899.54(C=O), 1723.42(C=O), 1619.48(C=C), 1433.27(CH_3)3542.23(O-H)	--------	---------

Antimicrobial Screening:

Antibacterial activity:(µg /ml)

Compounds	Gram +ve		Gram -ve	
	Staphylococcus Aureus	***Bacillus Subtilis***	***Klebsiella Pneumoniae***	***Escherichia Coli***
5 a	125	125	62.5	62.5
5 b	250	125	62.5	125
5 c	250	500	16.12	125
5 d	500	250	500	500
5 e	250	250	62.5	125
5 f	62.5	250	16.12	125
5 g	250	250	62.5	250
Ciprofloxacin	2	2	1	2
Norfloxacin	2	2	1	2

Minimum Inhibitory Concentration (MIC) values of the synthesized compounds.

Antifungal activity:(µg/ml)

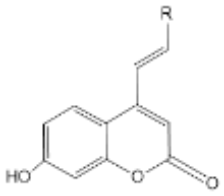
Minimum Inhibitory Concentration (MIC)of synthesized compounds are given below.

Compounds	***Candida Albicans***	***Aspergillus Niger***	***AspergillusFlavus***
5 a	4.03	4.03	2
5 b	1	8.06	4.03
5 c	1	16.12	8.03
5 d	1	8.06	8.03
5 e	1	8.06	4.03
5 f	1	2	2
5 g	1	4.03	4.03
Fluconazole	1	1	1
Griseofulvin	1	1	1

5.1 Physical and Spectral data:

Table no. 1.

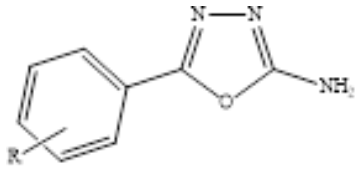
Comp.No.	**R**	**Nature of the Compound**	**M.P.(ºC)**	**Yield (%)**
3c	2-Cl	white crystalline powder	173-176	65
3d	2-Br	white crystalline powder	196-198	75



Recrystallization Solvent : Ethanol.

Table no. 2. **Physical data 4(a-j)**

Comp. No.	**R**	**Nature of the compound**	**M.P.(ºC)**	**Yield (%)**
4a	2,3-Cl	White crystals	222-224	65
4b	2,6-Cl	White crystalline powder	174-178	80
4c	2-Cl	White crystalline powder	165-168	85
4d	2-Br	White crystalline powder	170-73	85
4e	3-Br	White crystalline powder	198-200	80
4f	2-OCH_3	White crystalline powder	175-178	75
4g	3-OCH_3	White crystalline powder	180-183	70
4h	2-CH_3	White crystalline powder	165-168	75
4i	3-CH_3	White crystalline powder	182-85	80
4j	2-NO_2	White crystalline powder	192-95	75

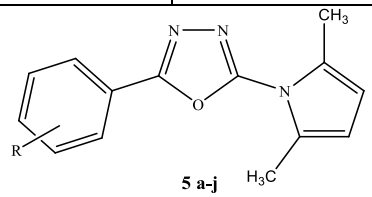


4 a-j

Recrystallization Solvent: Ethanol.

Table no. 3. **Physical data of 2-(3,5-dimethyl-1*H*-pyrrol-1-yl)-5-substituted-1,3,4-oxadiazoles (5 a-j)**

Comp.No.	R	Nature of the compound	Molecular Formula	Molecular Weight	M.P.(ºC)	Yield (%)
5a	2,3-Cl	Brown	$C_{14}H_{11}C_{l2}N_3O$	308	184-186	88
5b	2,6-Cl	Yellowish brown	$C_{14}H_{11}C_{l2}N_3O$	308	216-218	72
5c	2-Cl	Yellowish	$C_{14}H_{12}ClN_3O$	273	110-112	84
5d	2-Br	Yellowish	$C_{14}H_{12}BrN_3O$	318	240-242	57
5e	3-Br	Yellowish	$C_{14}H_{12}BrN_3O$	318	210-212	53
5f	2-OCH_3	Yellowish white	$C_{15}H_{15}N_3O_2$	269	144-148	56
5g	3-OCH_3	Brown	$C_{15}H_{15}N_3O_2$	269	142-145	69
5h	2-CH_3	Yellowish	$C_{15}H_{15}N_3O$	253	220-226	48
5i	3-CH_3	Yellowish white	$C_{15}H_{15}N_3O$	253	190-194	71
5j	2-NO_2	Blackish brown	$C_{14}H_{12}N_4O_3$	284	126-128	57

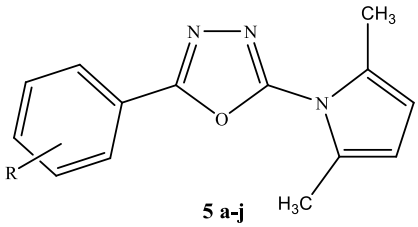


5 a-j

Recrystallization Solvent: Ethanol

Table no. 6. **Chromatographic data of 2-(3,5-dimethyl-1*H*-pyrrol-1-yl)-5-substituted-1,3,4-oxadiazoles (5 a-j)**

Comp. No.	R	$R_f$value
5a	2,3-Cl	0.71
5b	2,6-Cl	0.63
5c	2-Cl	0.68
5d	2-Br	0.58
5e	3-Br	0.72
5f	2-OCH_3	0.47
5g	3-OCH_3	0.42
5h	2-CH_3	0.66
5i	3-CH_3	0.49
5j	2-NO_2	0.77

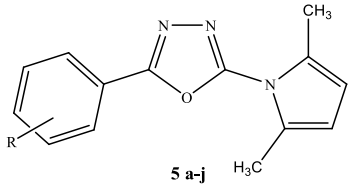


5 a-j

Silica Gel was used as stationary phase and iodine vapours as visualizing agent.

Solvent System Used: Ethyl acetate:N-Hexane and Ratio (7:3)

Table no. 9. **Spectral data of 2-(3,5-dimethyl-1*H*-pyrrol-1-yl)-5-substituted-1,3,4-oxadiazoles (5 a-j)**



5 a-j

Comp. No.	**R**	**IR spectra (KBr cm^{-1})**	**^{1}H-NMR spectra (δ, ppm)**	**Mass Spectra (m/z value)**
5a	2,3-Cl	1685.0, 1655.9 (C=N), 3005.9 (C-H; aromatic), 3326.1, 3260.1 (NH_2). 1073.9 (C-O-C)	----------	308
5b	2,6-Cl	1635.5 (C=N), 2922.2,2961.5 (C-H; aromatic), 3440.0 (OH), 3253.3 (NH_2), 1059.8 (C-O-C)	4.41-4.53 (2H,d,NH_2),10.17(1H,S,OH) 7.56,7.64,7.82 (8H, m, aromatic protons),.	308
5c	2-Cl	1639.62 (C=N), 3025.21 (C-H; aromatic), 3437.96, 3199.81 (NH_2) , 1071,48 (C-O-C)	----------	273
Comp. No.	R	**IR spectra (KBr cm^{-1})**	**^{1}H-NMR spectra (δ, ppm)**	**Mass Spectra (m/z value)**
5d	2-Br	1684.51 (C=N), 3010.44 (C-H; aromatic), 3329.47, 3258.08 (NH_2), 973.52 (C-O-C)	3.84, 4.00, 4.12, 4.19, 4.53 (4H,d,NH_2), 7.62,7.67,7.83,7.89,7.96 (8H, m, aromatic protons),.	318
5e	3-Br	1611.56 (C=N), 2938.56.0 (C-H; aromatic), 3421.05, 3187.37 (NH_2), 1009.08 (C-O-C)	4.03-4.11(2H,d,NH_2), 6.29-6.34(2H,d,CH), 7.28, 7.42, 7.54, 7.76, 7.85 (9H, m, aromatic protons),.	318
5f	2-OCH_3	1691.22 (C=N), 3014.98 (C-H; aromatic), 1073.9 (C-O-C).	--------	269
5 g	3-OCH_3	1613.47, 1545.68 (C=N), 3048.86 (C-H; aromatic), 3443.30 (OH), 1068.38 (C-O-C)	---------	269

Comp. No.	R	**IR spectra (KBr cm^{-1})**	**^{1}H-NMR spectra (δ, ppm)**	**Mass Spectra (m/z value)**
5h	2-CH_3	1600.83 (C=N), 2831.07 (C-H; aromatic), 3258.08(NH_2) , 1084.98 (C-O-C)	---------	253
5i	3-CH_3	1678.19 (C=N), 3010.61 (C-H; aromatic), 3335.86,(NH_2), 940.98 (C-O-C)	---------	253
5j	2-NO_2	1691.06 (C=N), 2924.38 (C-H; aromatic), 1084.20 (C-O-C)	6.29-6.34 (2H d CH), 7.11, 7.45, 7.96, 8.53 (9H, m, aromatic protons),.	284

5.2 Microbiological Screening Antibacterial Activity:

Table No.10 **MIC (µg /ml) values of the synthesized compounds 5(a-j)**

Compounds	***Candida Species***	***Aspergillus Species***	
	Candida Albicans	***Aspergillus Niger***	***AspergillusFlavus***
5a	4.03	4.03	2
5b	1	8.06	4.03
5c	1	16.12	8.03
5d	1	8.06	8.03
5e	1	8.06	4.03
5f	1	2	2
5g	1	4.03	4.03
5h	4.03	16.12	16.12
5i	2	4.03	16.12
5j	2	1	1
Fluconazole	**16**	**8**	**8**
Griseofulvin	**500**	**100**	**100**

Antifungal Activity: ***Table No.11*** **MIC (µg /ml) values of the synthesized compounds 5(a-j)**

Compounds	Gram +ve		Gram –ve	
	Staphylococcus Aureus	***Bacillus Subtilis***	***KlebsiellaPneumoniae***	***Escherichia Coli***
5a	125	125	62.5	62.5
5b	250	125	62.5	125
5c	250	500	16.12	125
5d	500	250	500	500
5e	250	250	62.5	125
5f	62.5	250	16.12	125
5g	250	250	62.5	250
5h	125	500	62.5	250
5i	500	250	125	250
5j	250	500	250	500
Ciprofloxacin	**2**	**2**	**1**	**2**
Norfloxacin	**2**	**2**	**1**	**12**

Comparative MIC Chart of the Synthesized Compounds 5(a-j)

Antibacterial Activity of Compound 5a:

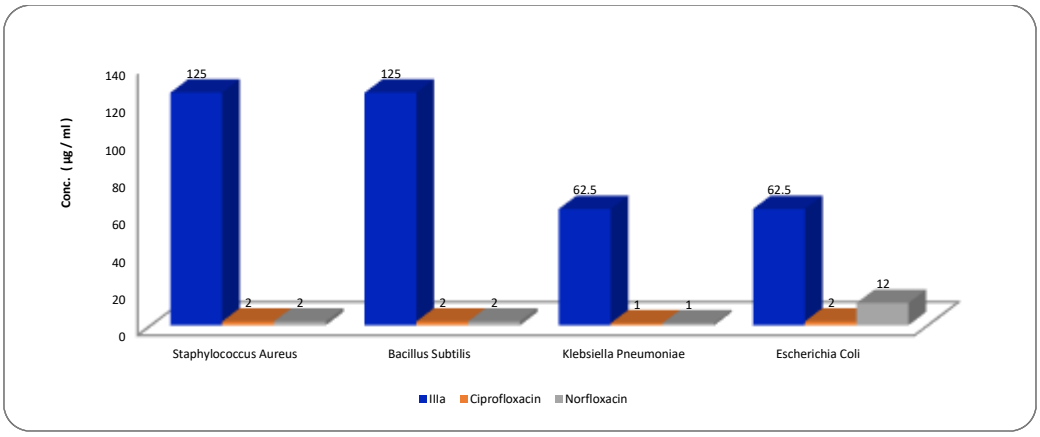


Chart No. 01

Antifungal Activity of Compound 5a:

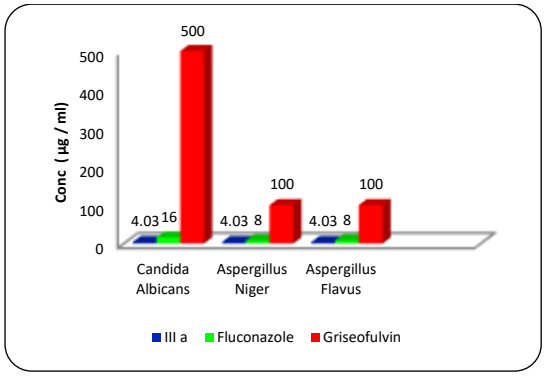


Chart No. 02

Antibacterial Activity of Compound 5b:

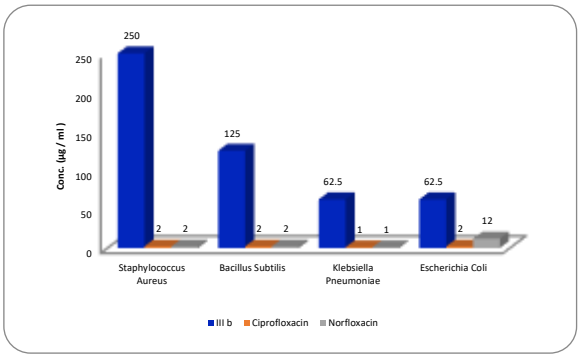


Chart No. 03

Antifungal Activity of Compound 5b:

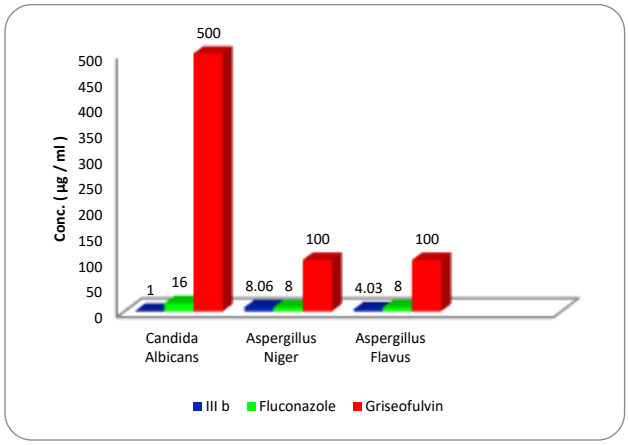


Chart No. 04

Antibacterial Activity of Compound 5c:

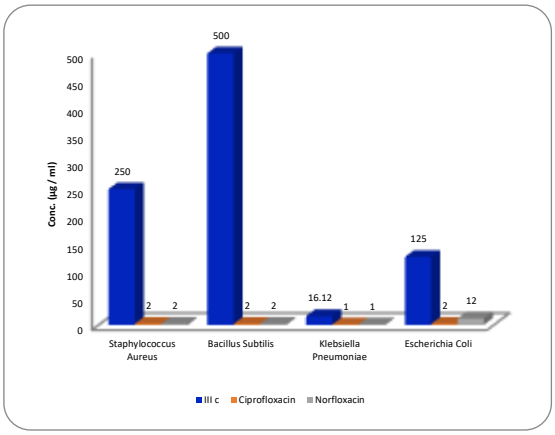


Chart No. 05

Antifungal Activity of Compound 5c:

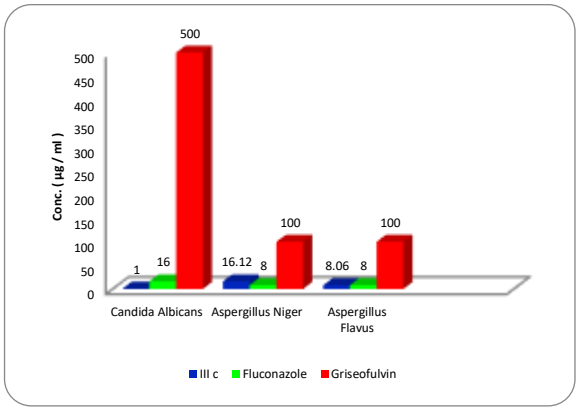


Chart No. 06

Antibacterial Activity of Compound 5d:

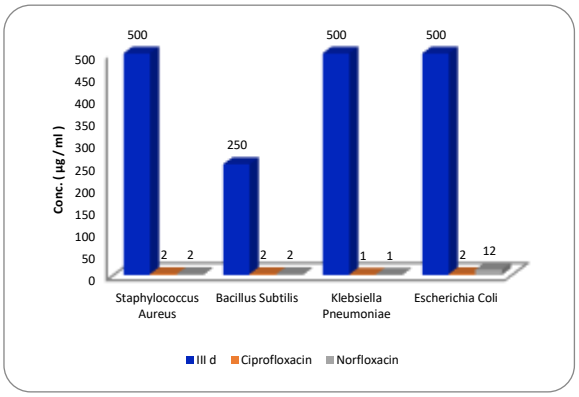


Chart No. 07

Antifungal Activity of Compound 5d:

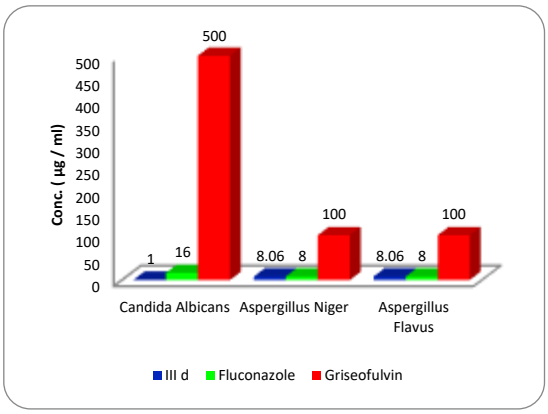


Chart No. 08

Antibacterial Activity of Compound 5e

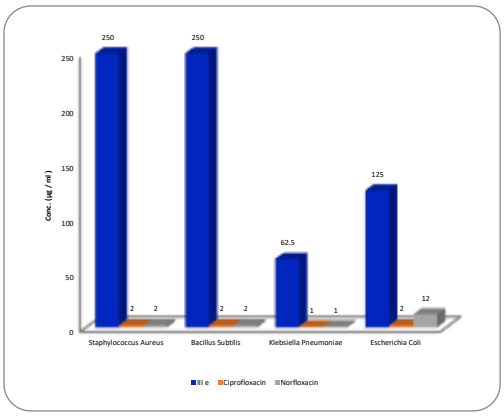


Chart No. 09

Antifungal Activity of Compound 5e:

Antibacterial Activity of Compound 5f:

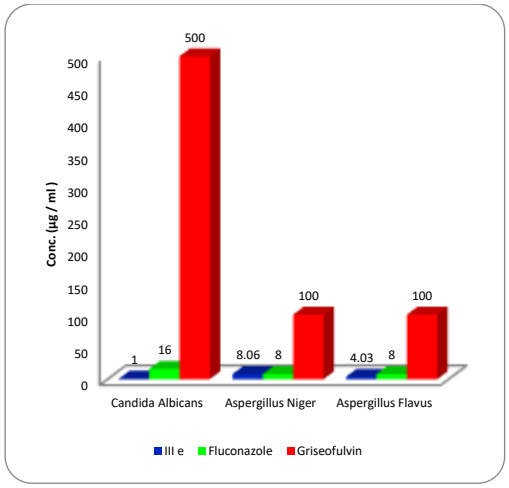


Chart No. 10

Antifungal Activity of Compound 5f:

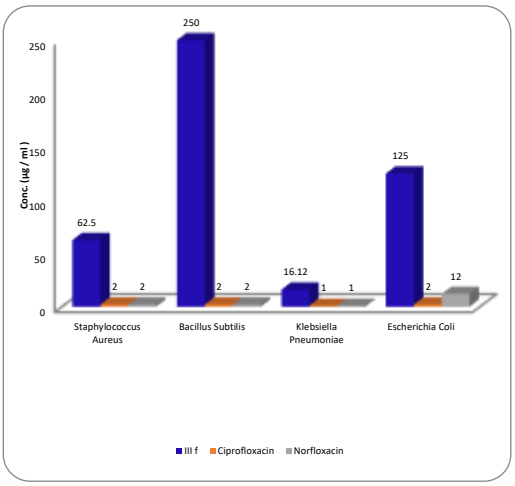


Chart No. 11

Antibacterial Activity of Compound 5g:

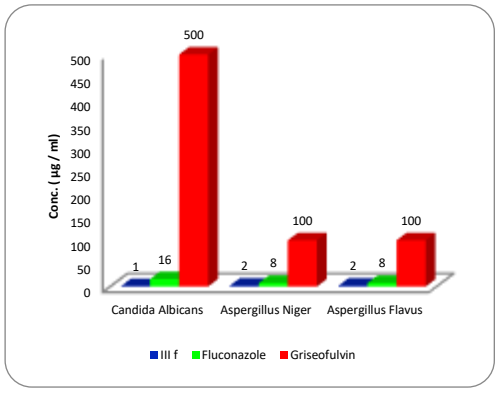


Chart No. 12

Antifungal Activity of Compound 5g:

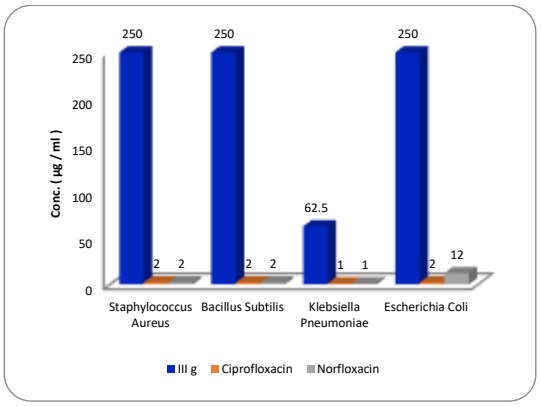


Chart No. 13

Antibacterial Activity of Compound 5h:

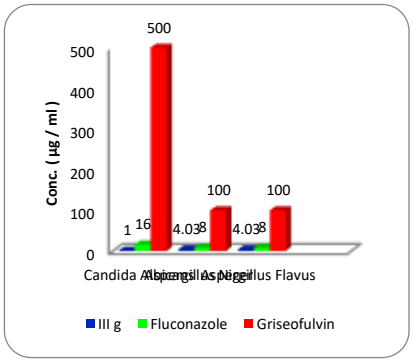


Chart No. 14

Antifungal Activity of Compound 5h:

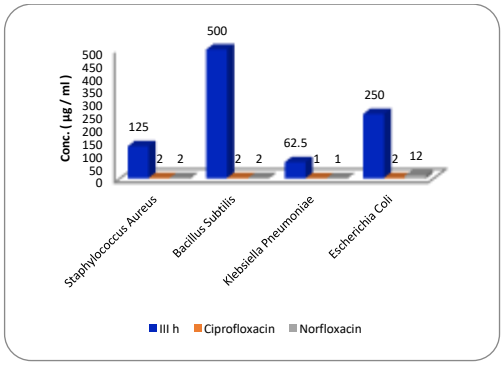


Chart No. 15

Antibacterial Activity of Compound 5i

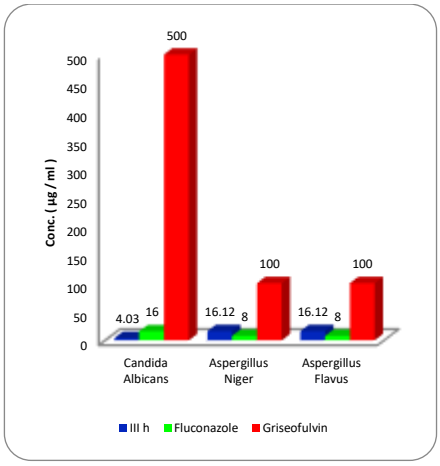


Chart No. 16

Antifungal Activity of Compound 5i:

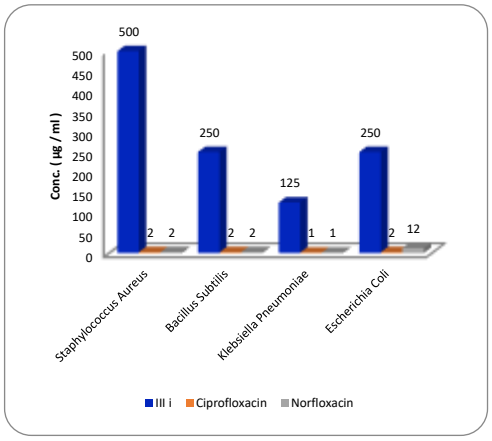


Chart No. 17

Antibacterial Activity of Compound 5j:

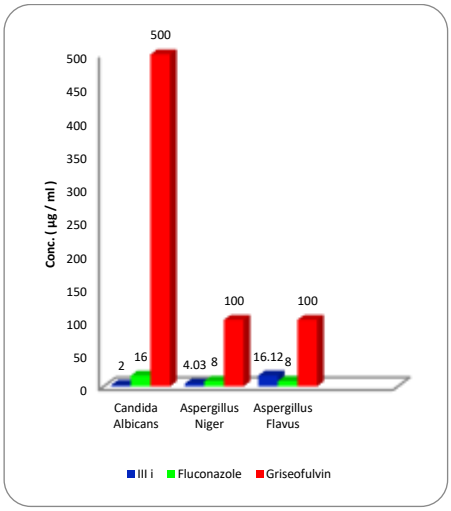


Chart No. 18

Antifungal Activity of Compound 5j:

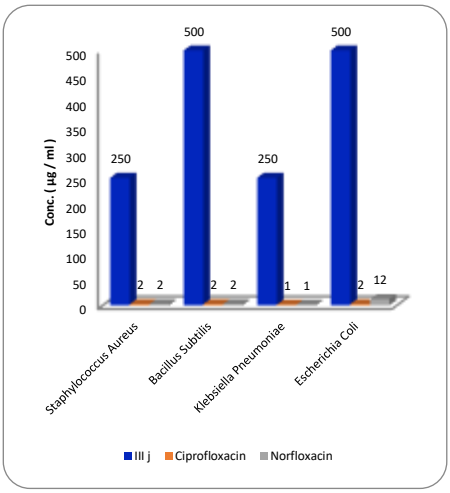


Chart No. 19

Microbiological Screening: Study of MIC Compound 5c: KlebsiellaPneumoniae

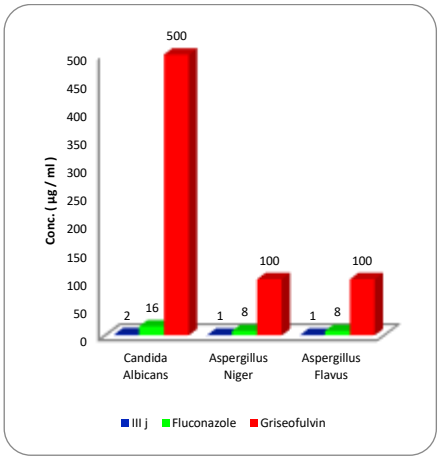


Chart No. 20

Figure No.01

Compound 5f: KlebsilleaPneumoniae **Compound 5f: Staphylococcus Aureus** **Compound 5f: Candida Albicans**

Figure No.02

Compoun 5j Aspergillus Niger

Figure No.03

Compound 5f: AspergillusFlavus

Figure No.04

Figure No.05

Figure No.06

6.1 Summary

pyrazole derivatives were synthesized via cyclization of various hydrazides with ethyl acetoacetate in acetic acid. Structural confirmation was achieved using NMR, IR, and mass spectrometry. IR spectra displayed characteristic peaks such as N–H (3072.51 cm^{-1}), C=O (1612.02 cm^{-1}), C–N (1277.27 cm^{-1}), aromatic C–H (2857.57 cm^{-1}), aliphatic C–H (2952 cm^{-1}), aromatic C–C (1433.31 cm^{-1}), and C–O–C (1052.14 cm^{-1}), verifying the functional groups present. Mass spectra gave m/z values of 404 (5a), 314 (5b), and 335 (5d).

Antimicrobial activity was assessed by the broth microdilution method using Mueller-Hinton agar. Antibacterial testing against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* revealed moderate to poor inhibition overall. Compounds 5c (–OCH_3) and 5f (–Br) exhibited notable activity at 16.12 µg/mL against *Klebsiella pneumoniae*. Antifungal evaluation against *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* showed that 5b (–CH_3), 5c (–OCH_3), and 5d (–F) were active at 1 µg/mL against *C. albicans*, while 5f (–Br) was active at 2 µg/mL against all three tested fungi.

These results suggest that substituent variation in pyrazole derivatives influences antimicrobial profiles, with certain electron-donating and halogen groups enhancing specific antibacterial or antifungal activities.

6.2 Conclusion:

Above mentioned research work confirms the potential of chromone based pyrazoline derivatives as need for development of novel and better compounds possessing excellent biological activities. In conclusion, with proper designing and structure activity relationship studies of compounds having chromone based pyrazoline nucleus, prospective compounds can be synthesized for a particular biological activity.

The complete exploitation of chromone based pyrazoline lead may be expected from the scientific community, to discover the safe, potent drug candidates with lesser side effects.

References

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