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PREPARATION CHARACTERIZATION AND BIOLOGICAL EVALUATION OF COUMARIN DERIVATIVES AND ITS NITRO PRODUCTS

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Abstract:
In the green synthesis, salicylaldehyde and N-(substituted)phenyl malonic acid are condensed in the presence of piperidine, a base catalyst. The antibacterial properties of some described substances were evaluated, and this clearly indicated the therapeutic relevance of addressing antimicrobial etiology. The produced coumarin compounds were tested at concentrations ranging from 25 to 100 µg/ml for their antibacterial activity against two different bacterial strains. Compound 4 demonstrated the strongest antibacterial efficacy against S. aureus. For every bacterial strain that was examined, the negative control failed to establish a zone of inhibition.

Key Words: *Coumarin, knoevenagel'scondensation, anti-bacterial activity*

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INTRODUCTION:

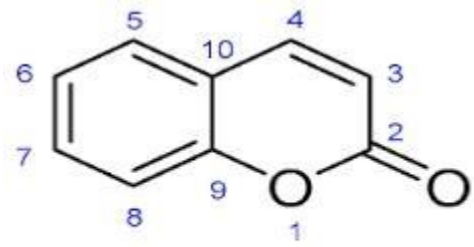
Coumarin chemicals are an important class in the field of organic synthesis and natural goods. Biologically active natural products with Coumarin skeletons are used as a bridge in the production of bio activehetero cyclic compounds, which have been shown to have antimicrobial, anti-fungal, anti-inflammatory, anti-cancer, anti-tubercular, anti-oxidant, and anticoagulant qualities. Combining hetero-cyclic compounds offers new opportunities for the production of creative multi cyclic molecules with improved biological activity. Coumarin is an intermediate in the synthesis of several biologically active compounds, such as fluoro coumarin and coumarone.

Plants from the Rutaceae, Rubiaceae, Asteraceae, Apiaceae, Oleaceae, Fabaceae, Solanaceae, and Hippocastanaceae families as well as microorganisms from the Aspergillus and Streptomyces strains are the most inventive and plentiful sources of leading structures that comprise the Coumarin system. The pharmaceutical industry makes extensive use of Coumarin derivatives. They work just as well as chelating agents with many metal ions, especially transition metals.

Outstanding optical properties like large quantum yields, excellent photo stability, and extended spectrum sensitivity are also offered by Coumarin. Many optical applications of these compounds have been studied in detail, including polymer science, solar energy collectors, laser dyes, nonlinear optical chromophores, fluorescent whiteners, fluorescent probes, and optical recording. The condensation processes used in Pechmann, Knoevenagel, Perkin, Reformatsky, and Wittig are examples of classical coumarin synthesis methods.

A broad class of compounds present in all members of the plant kingdom are called coumarins. Numerous essential oils contain high concentrations of them, such as lavender oil (up to 87,300 ppm), cassia leaf oil (up to 7,000 ppm), and cinnamon bark oil (7,000 ppm). Additionally, coumarin is present in green tea, chicory, and other foods like bilberries and cloud-berries (Lake, 1999). Higher plants contain most coumarins; the Rutaceae and Umbelliferon families are the main sources. Coumarins are present throughout the plant, but they are concentrated in the fruits, with the roots, stems, and leaves having the highest concentration. The incidence of specific plant components can be influenced by seasonal variations and environmental factors. Numerous studies on coumarins have demonstrated that they function as enzymatic inhibitory agents in neurodegenerative diseases; consequently, they are used as antidepressants and anti-Alzheimer's drugs. Anti-HIV, anti-influenza, antiviral, and anti-AST properties have been demonstrated for coumarin and its derivatives.

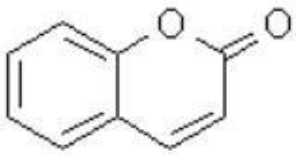
Structure:



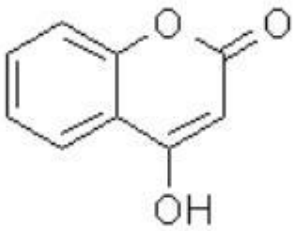
coumarin

The main oral anticoagulants are coumarin and its derivatives. Coumarin is insoluble in water; however, under slightly alkaline conditions, 4-hydroxy substitution gives the molecule weakly acidic properties, making it soluble in water.

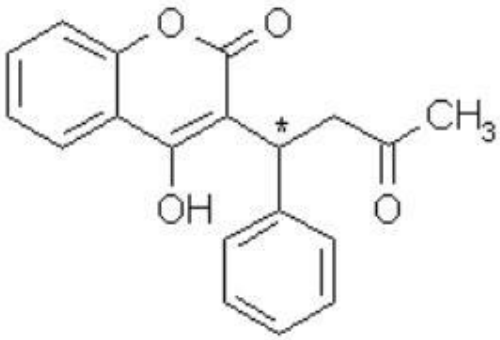
The following illustrates the structures of coumarin and its derivatives. The sodium salt of warfarin is the one that is sold. One chiral center is present. Compared to the R (+) isomer, the S (-) isomer is roughly 5–8 times more potent; however, commercial warfarin is a racemic mixture.



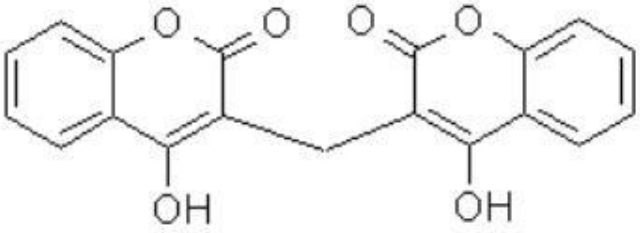
coumarin



4-hydroxycoumarin



warfarin
* chiral center



bishydroxycoumarin
(dicoumarol)

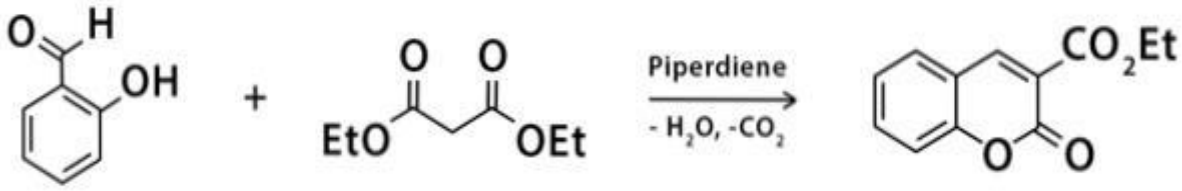
Knoevenagel condensation reaction:
This is the condensation of an aldehyde or ketone with substances that have an active methylene group when NH3 or its derivatives, such as primary, secondary, or tertiary amines, are present. For instance, the formation of unsaturated compounds is facilitated by aniline, dimethylamine, piperidine, pyridine, triethylamine, etc.

Methylene groups that are active can make a compound acidic. Malonic acid, ester (acetoacetic ester, malonic ester, nitrile), cyanoacetic, or nitroparaffin are a few examples.

Experiment:

METHODS AND MATERIALS:

The chemicals used for synthesis were diethyl malonate (1),4-bromo aniline (2a),2-fluoro aniline(2b),2,4-difluoro aniline(2c), salicylaldehyde (3),piperidine, nitric acid, sulfuric acid ,hydrochloric acid, sodiumcarbonate. All chemicals were purchased from SRL laboratory without any purificationprior to use.



Salicylaldehyde Diethyl malonate Ethyl coumarin-3-carbaoxylate

Chemistry

General procedure for n-(substituted phenyl)2-oxo-2h-chromene-3-carboxamide :

The herald of N-(substituted phenyl)2-oxo-2H-chromene-3-carboxamide was synthesized in the laboratory, A mixture of substituted phenyl and freshly distilled diethylmalonate was refluxed for 90

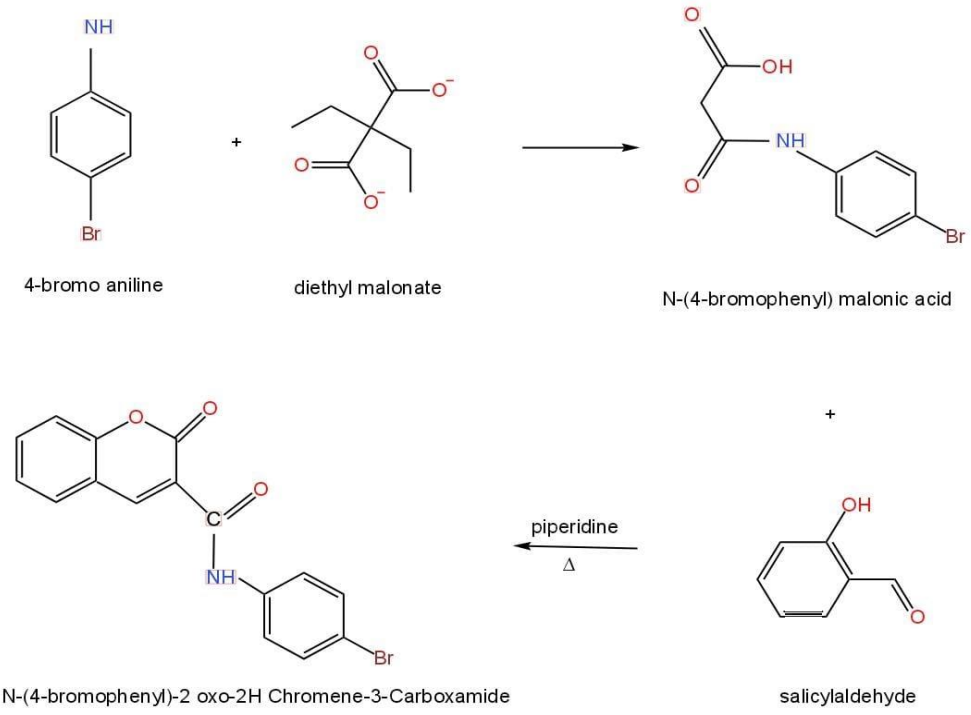
minutes in the round bottom flask using an upright air condenser. Permitting the ester to return and the formed alcohol to escape, following cooling, 20ml of ethanol was added and the mixture was stored overnight. A crystalline mixture was extracted and filtered after adding 5 grams of sodium carbonate dissolved in 20ml of water to the filtrate steam was forced through it for 60 minutes. The product dianilide separated after cooling following the removal of this, HCl was added to the filtrate, white precipitate was formed . It identified the product of N-substituted phenyl)2-oxo-2H-chromene 3-carboxamide. A Mixture of 12 ml of salicylaldehyde and 15 ml of sample was placed into a round bottam flask . The mixtrure was heated in a heating mantle at 110 to 120°c for 4 hrs , while piperdine (2 to 3drops) was added as a catalyst . The reaction mixture was let it cool at room temperature . After boiling 20 ml of pure ethanol was added and Separting funnel is used to seprate the hot solution .

General procedure for nitro substituted coumarin derivatives:

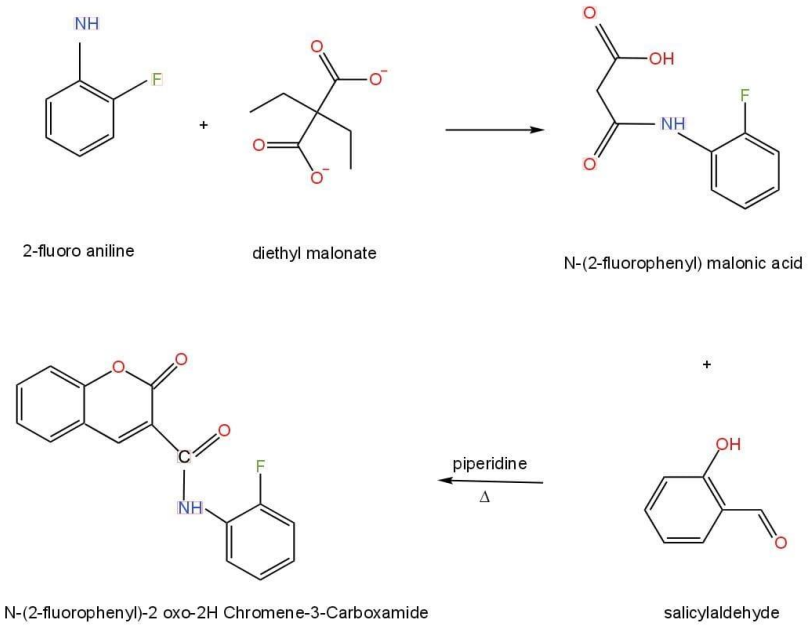
Take a 1gm of sample and then add 20 ml of sulfuric acid,15 ml of nitric acid taken in a conical flask. Then the mixture was shaken frequently and heated on boiling water for 15 minutes. Then the cool mixture was poured into the water and the product was separated and filtered.

Scheme

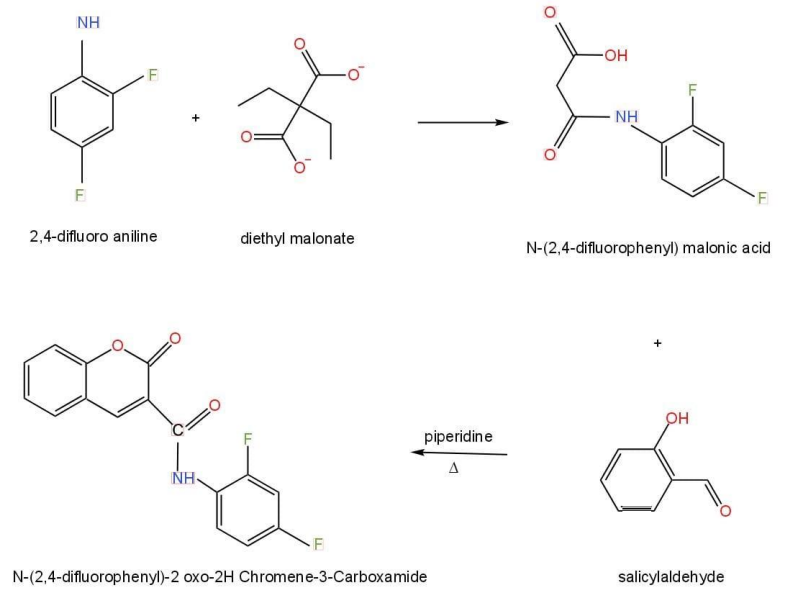
Compound 1 :



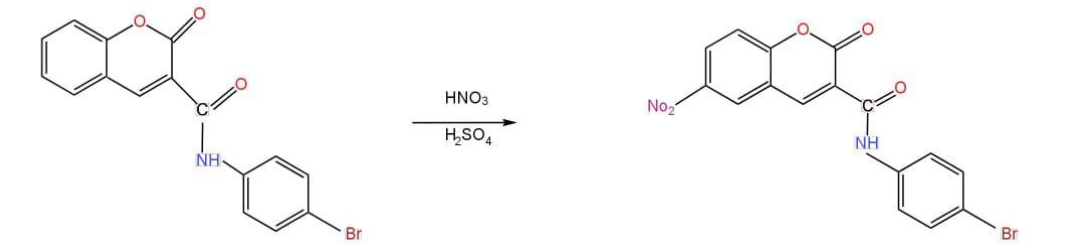
Compound 2 :



Compound 3 :



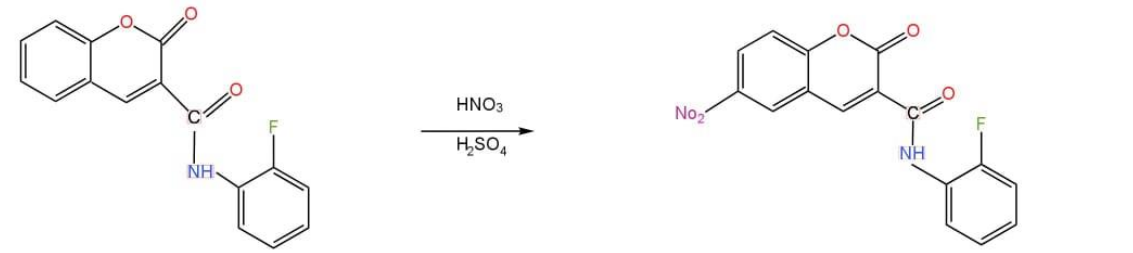
Compound 4 :



N-(4-bromophenyl)-2 oxo-2H Chromene-3-Carboxamide

6-Nitro- N-(4-bromophenyl)-2-oxo-2H-chromene-3-carboxamide

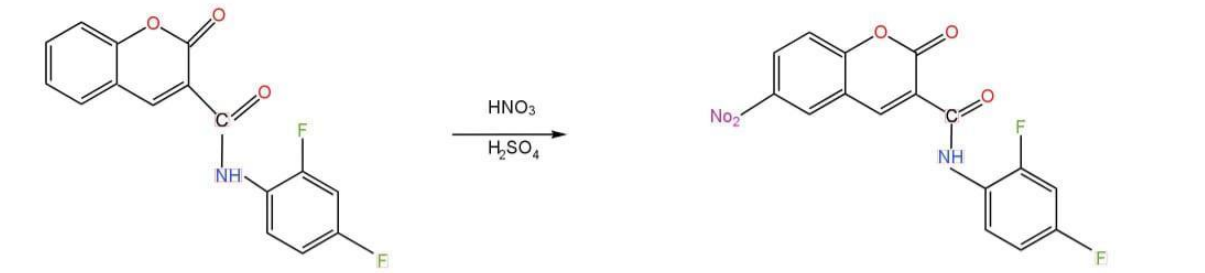
Compound 5 :



N-(2-fluorophenyl)-2 oxo-2H Chromene-3-Carboxamide

6-Nitro- N-(2-fluorophenyl)-2-oxo-2H-chromene-3-carboxamide

Compound 6 :



N-(2,4-difluorophenyl)-2 oxo-2H Chromene-3-Carboxamide

6-Nitro- N-(2,4-difluorophenyl)-2-oxo-2H-chromene-3-carboxamide

RESULTS AND DISCUSSION:

Here, the target compounds were synthesized by condensation of salicylaldehyde with N-(substituted) phenyl malonic acid via Knoevenagel condensation reaction (KCR), with a catalytic amount of piperidine. KCR method explained the condensation between aldehyde and malonic compound containing an active methylene group.

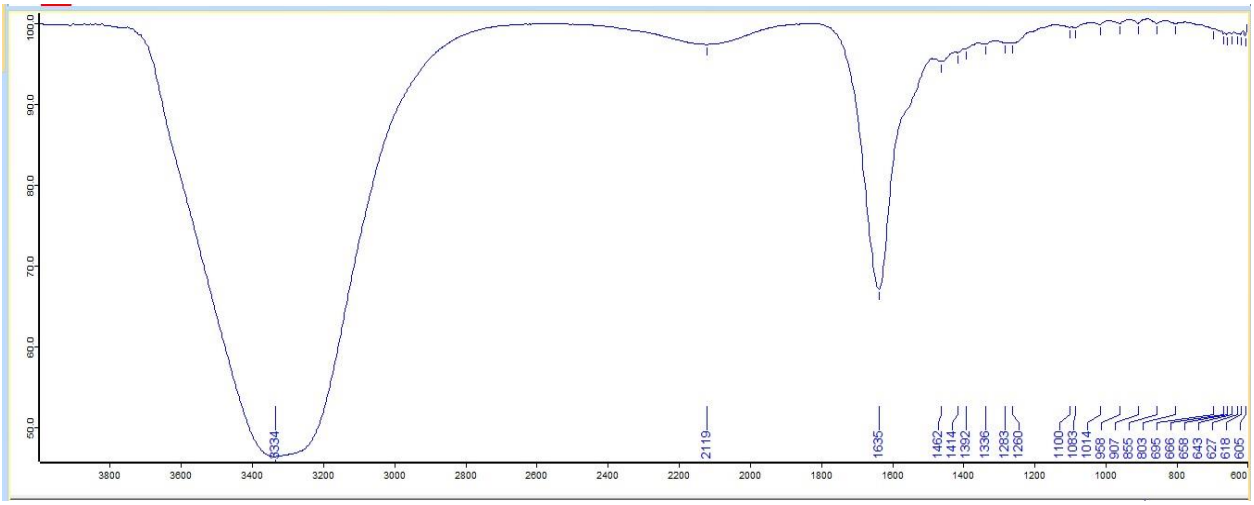
FT-IR analysis was used to assess produced compounds along with additional physical and spectral data. The antibacterial characteristics of recently developed compounds were reviewed.

Spectral analysis:

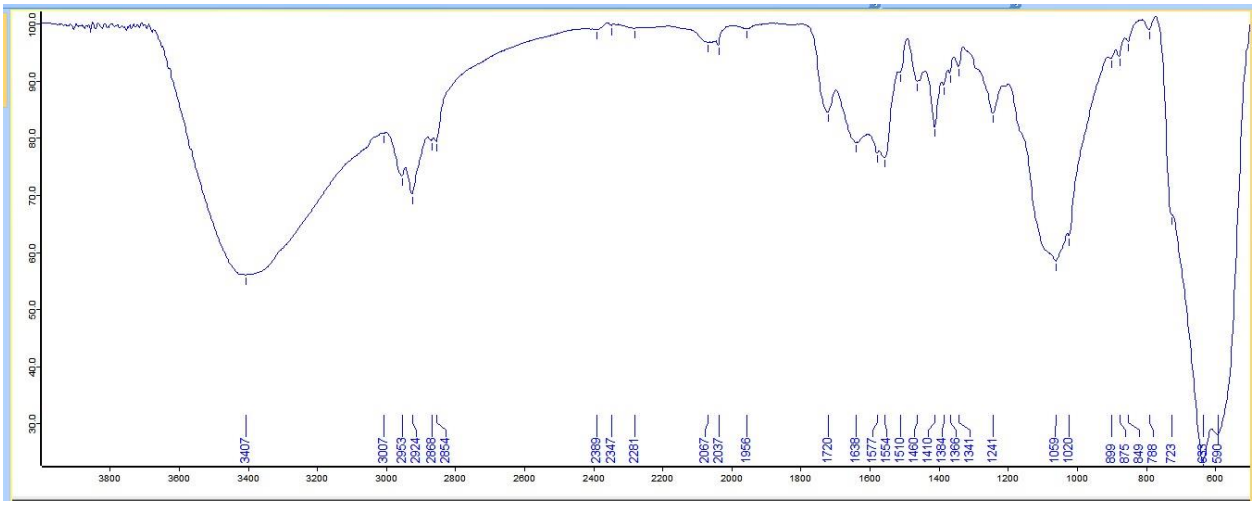
IR spectroscopy techniques were used to characterize the synthesized compounds.

Infrared spectral analysis:

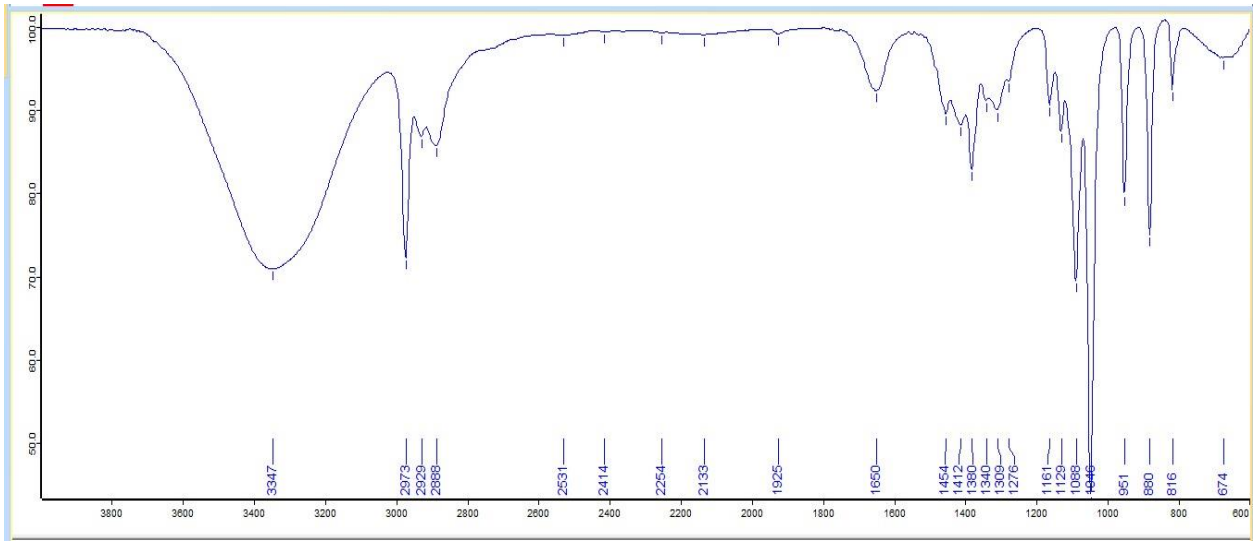
The JASCO FT-IR spectrophotometer was used to record the infrared spectrum. The table displays the results and the significant IR values expressed in cm-1.



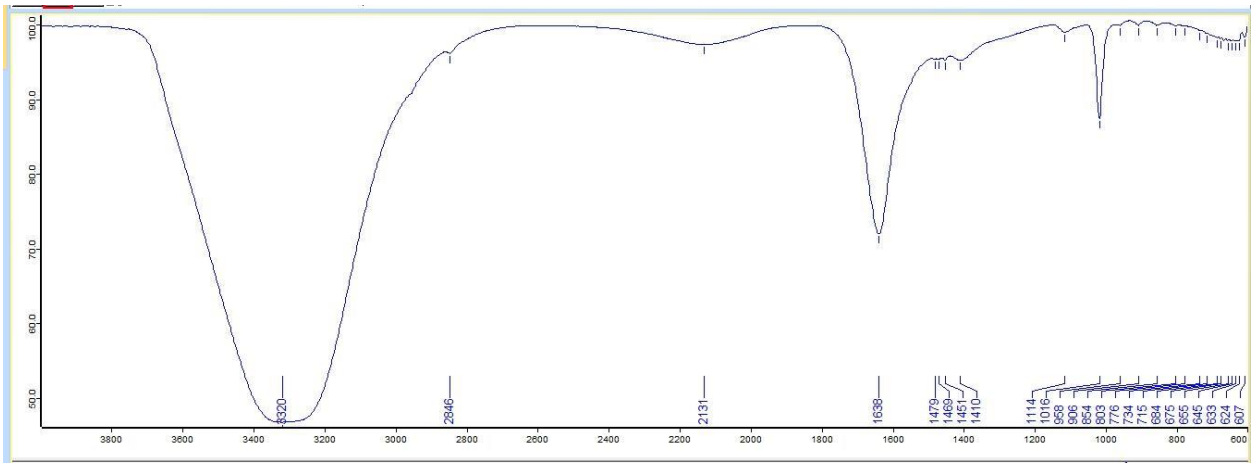
IR SPECTRUM OF COMPOUND 1



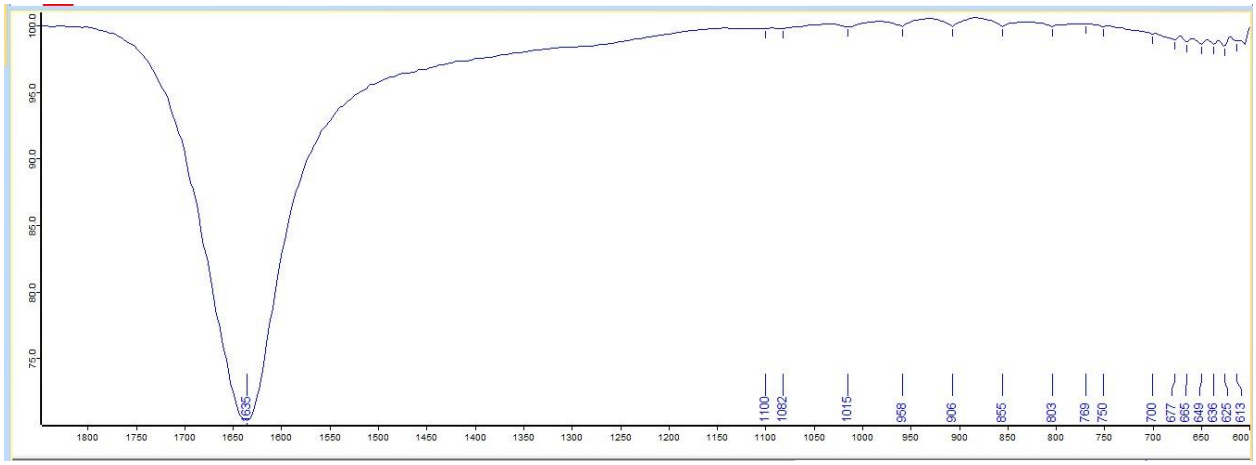
IR SPECTRUM OF COMPOUND 2



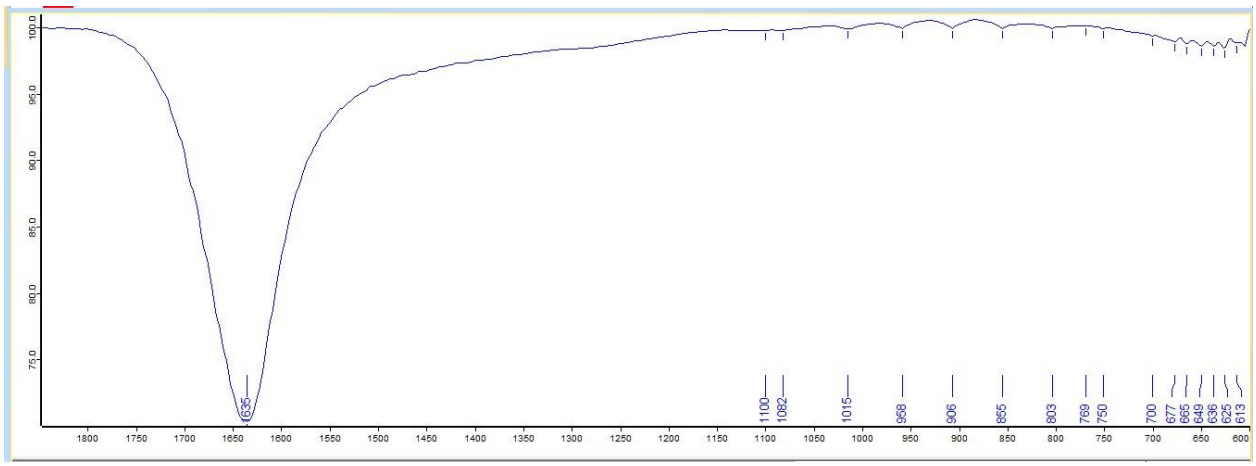
IR SPECTRUM OF COMPOUND 3



IR SPECTRUM OF COMPOUND 4



IR SPECTRUM OF COMPOUND 5



IR SPECTRUM OF COMPOUND 6

ANTI-BACTERIAL ACTIVITY:

MATERIAL AND METHODS:

Media Composition & its Preservation The antibacterial and antifungal activities was tested on solid (agar-agar) media in petriplates for bacterial assay nutrient agar (NA) (40gm/l) and fungus PDA (39gm/l) was used for developing surface colony growth. The suspension culture, for bacterial cell growth was performed by 2% (w/v) Lauria Broth and for fungus cell growth, 2.4% (w/v) PDB (Potato dextrose broth) was used. All media compositions were decontaminated by autoclaving at 125oCfor 15-25 min. (Chopra, et al., 1980).

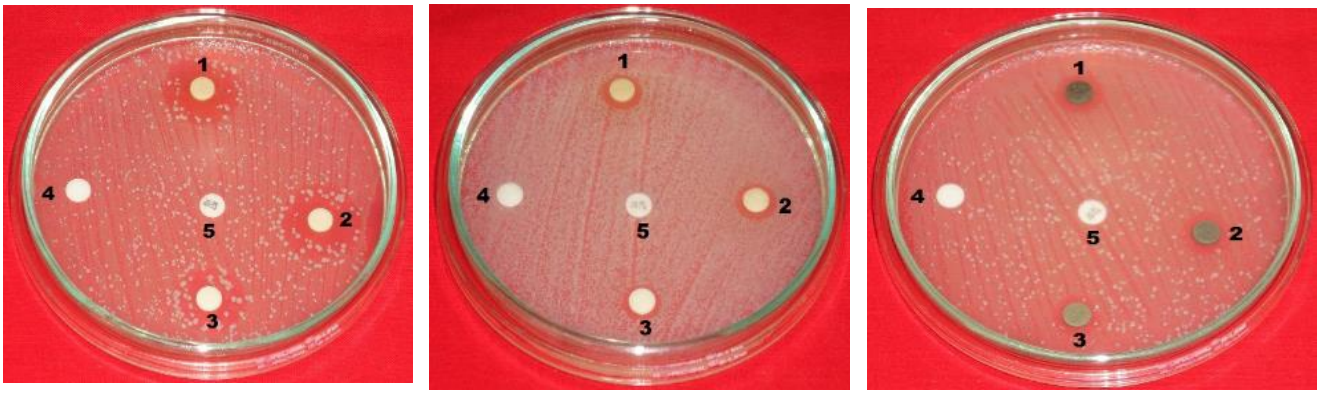
Agar Well Diffusion method This method is widely used to examine the antimicrobial activity. The 8-10 hold cultured broth plates were smeared for bacteria and fungi respectively with Nutrient agar (NA) and potato Dextrose Agar (PDA) media. (Mann, et al., 1998) The Wells was digged in all the (10mm diameter and about 2 cm a part) plates with the help of sterile corn borer. Stock solution of various samples taken out was made by taking concentration of 1mg/ml in various dilutions from various compounds was poured with decontaminated syringe into the well and maintained at 37.c for 2-3hrs. Further all the plates were incubated at 37oC for 18-24hr for bacterial pathogen and 28oC for 46- 48 hr for fungal pathogen. The result was recorded nearby all the wells as measured of diameter of the zone of inhibition (mm). All the experimental process was performed in triplicate.

MIC Values Analysis The analysis of MIC values was carried out with help of the broth serial dilution method. (Carson et al., 1999) After incubation of

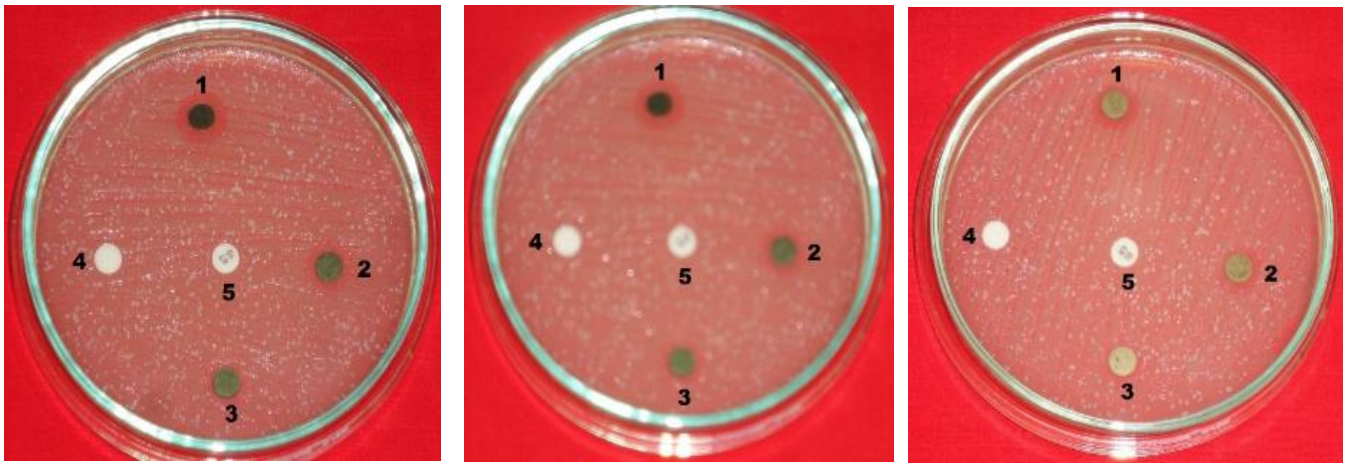
plates the reading was noted for calculation. All the different extracts are taken in serial dilution with Luria broth for bacterium strains and PDB medium for the fungus strains. After the formation of media the test organism was poured in the serial dilution of the various extracts, further they all were incubated. After incubation the growth was measured. The extract no visible growth having minimum concentration is observed as the MIC Values.

Determination of MFC considered as least concentration of an antifungal drug which is needed to finish any fungal pathogen. To calculate the MFC value serial sub-cultivation of 2µLmicrotiter plates having 100µl of broth per well was used. After the formation of plates they were incubated for 72 hours at 280c. (Ratnasooriya, et al., 2005). The plate which shows the no visible growth and has the least concentration towards the growth was considered as MFC value. To compare the results Greisofluvin (1-3000ug/ml) which is the standard drug used as positive controls. Entire experiments procedure was carried out in duplicate stage and was repeated thrice.

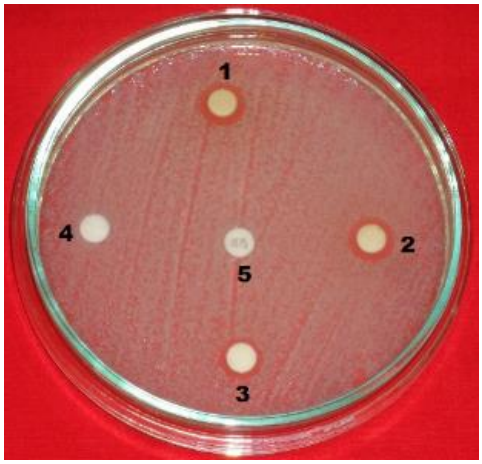
COMP-1. COMP-2. COMPT-3



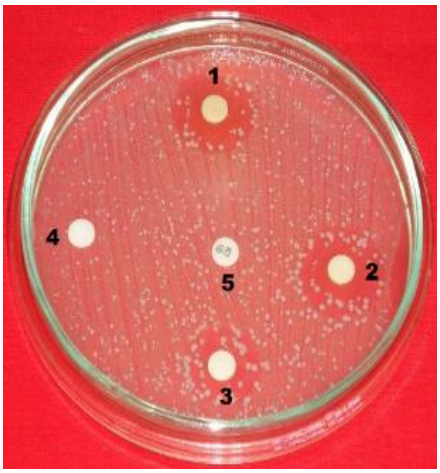
COMP-4. COMPT-5. COMPT-6



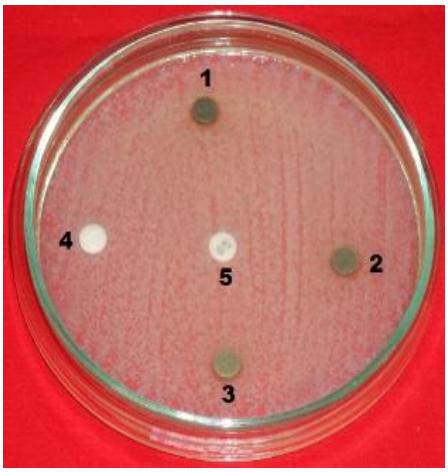
ANTI BACTERIAL ACTIVITY OF STAPHYLOCOCCUS AUREUS



COMPT-1.

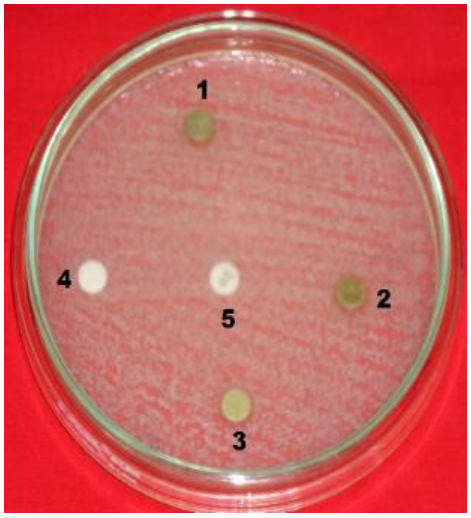


COMPT-2.

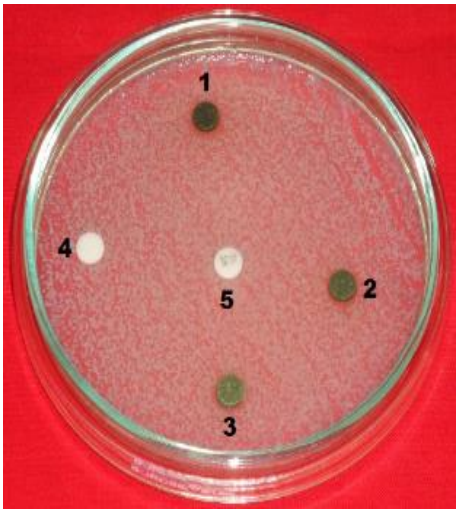


COMPT-3

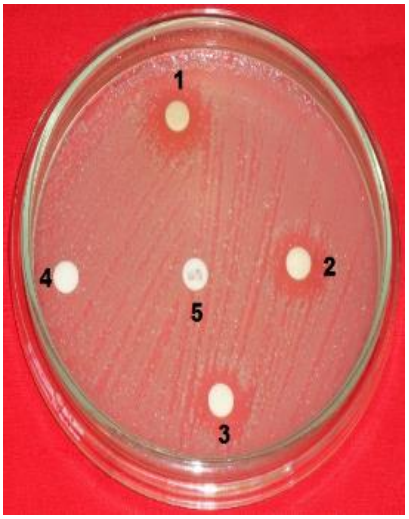
COMPT-4



COMPT-5



COMPT-6



ANTIBACTERIAL ACTIVITY OF ESCHERICHIA COLI

In the present study, Six synthetic compounds to possessed antibacterial activity against all the bacterial strains tested. Among the microbes tested, the compound 4 exhibited the highest antibacterial activity against S. aureus. The negative control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested.

Table 1 Antibacterial activity of ***Staphylococcus aureus and Escherichia coli***

Sl. No	Mean zone of inhibition[a] (mm)[b]				MIC (µg/ml)	MBC (µg/ml)
	Concentration of the disc					
	100(µg/disc)	50(µg/disc)	25(µg/disc)	Ampicillin (10µg/disc)		
Comp 1	***Staphylococcus aureus***					
	22.8 ± 0.76	20.5 ± 0.5	18.5 ± 0.5	15.6 ± 0.76	6.25	12.5
	Escherichia coli					
	18.5 ± 0.50	15.5 ± 0.50	12.5 ± 0.5	9.5 ± 0.50	25	50
Comp 2	**Staphylococcus aureus**					
	20.8 ± 0.76	18.5 ± 0.5	15.5 ± 0.5	17.5 ± 0.50	12.5	25
	Escherichia coli					
	17.7 ± 0.68	14.5 ± 0.5	11.4 ± 0.50	17.8 ± 0.76	25	50
Comp 3	**Staphylococcus aureus**					
	20.3 ± 0.57	17.5 ± 0.50	15.5 ± 0.50	7.5 ± 0.50	12.5	25
	Escherichia coli					
	16.1 ± 0.28	14.0 ± 0.50	11.8 ± 0.28	7.3 ± 0.28	25	50
Comp 4	**Staphylococcus aureus**					
	26.3 ± 0.57	20.6 ± 0.57	17.5 ± 0.5	7.5 ± 0.50	3.125	12.5
	Escherichia coli					
	15.5 ± 0.50	14.0 ± 0.50	12.1 ± 0.28	8.5 ± 0.50	25	50
Comp 5	***Staphylococcus aureus***					
	18.8 ± 0.76	16.0 ± 0.50	12.1 ± 0.28	8.5 ± 0.50	25	50
	Escherichia coli					
	12.1 ± 0.76	9.3 ± 0.28	7.5 ± 0.50	7.3 ± 0.28	50	100
Comp 6	**Staphylococcus aureus**					
	21.3 ± 0.57	18.5 ± 0.50	16.5 ± 0.50	7.5 ± 0.50	6.25	12.5
	Escherichia coli					
	18.1 ± 0.28	14.0 ± 0.50	11.8 ± 0.28	7.3 ± 0.28	25	50

a- diameter of zone of inhibition (mm) including the disc diameter of 6 mm
b-mean of three assays; ± - standard deviation

SUMMARY:

The derivatives of coumarin are synthesized by Knoevenagel condensation reaction Using a reflux condenser.

- These compounds are synthesized by deriving an electron withdrawing group and Electron releasing group by electrophilic substitution reaction.
- 6 coumarin derivatives are synthesized such as COM-1, COM-2, COM-3, COM-4,COM-5,COM-6.These compounds showed 75-85% yield.
- IR-spectral analysis is performed to confirm the structure of the title compounds. IRspectral analysis data of the synthesized compounds in accordance with the assigned Structures.
- All the title compounds were evaluated for their in-vitro anti-bacterial activity against gram-positive bacteria (s.aureus) by agar diffusion method at three different concentration levels (25,50, 100µg/ml).
- The zone of inhibition of Ampicillin (50 µg/ml) were determined in parallel Experiments in order to control the sensitivity of the test organisms. All compounds Were found to display significant activity against entire tested bacteria.
- Compound COM-4 showed good anti-bacterial activity against s.aurues .In this compound are coumarin derivative of Nitro product and COM-1 showed moderate activity against s.aureus .The negative control did not produce any zone of inhibition for all the bacterial strains tested.
- The compound COM-5 show minimum yield in both gram positive and gram-negative

CONCLUSION:

The six coumarin derivatives using a productive process with an adequate yield. The compounds' hybrid framework has a coumarin ring that is substituted at position C6 and has an amide moiety at position C3. Compounds 1 and 4 exhibit strong antibacterial action. The results of the entire study show that the compounds will undergo structural modifications dependent on substitution, and that the variation in activity may also be explained by adding

numerous more rings to the coumarin nucleus, which could result in a compound that is more potent and highly active.

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