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Synthesis, Structural Characterization, And Biological Assessment of Coumarin-Based Compounds for Antimicrobial Activity

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Abstract

The current paper is devoted to the synthesis, structural characterization, and the antimicrobial assay of the series of coumarin-based derivatives that are considered as the potential candidates of new antimicrobial agents. Synthesis of five coumarin analogs (C1-C5) was reproducibly carried out by following the usual organic synthesis procedures with yields between 68 and 89 percent with C3 and C4 having the highest synthetic efficiency. The presence of critical functional groups, such as the typical lactone C=O stretch and aromatic C=C bands had been confirmed by structural confirmation which was conducted by FTIR, NMR, and UV and visible spectroscopy with the overall confirmation accuracy of greater than 90%. The antimicrobial properties of the products of the synthesized compounds were determined with Minimum Inhibitory Concentration (MIC) assays against particular microbial strains and it was observed that there were drastic differences in potency of the compounds depending on the structural changes. Among the derivatives, C4 was the most active with the least MIC (31.25 µg/mL) and 100% relative potency, and then C3, which exhibited a high antimicrobial potential. The synthesis efficiency, structural validation, and biological performance are analyzed as a whole, which underlines the significance of the structure-activity relations in the development of efficient coumarin-based antimicrobial agents. Altogether, the research findings highlight the potential application of these derivatives as viable scaffolds to pursue pharmaceutical development against drug-resistant microbial pathogens in the future.

Keywords: Coumarin Derivatives, Structural Characterization, Antimicrobial Activity, FTIR, NMR, MIC Analysis.

1. INTRODUCTION

Coumarins form a substantial family of naturally occurring benzopyrone derivatives that are broadly spread out in plants, fungi and in microorganisms. Their peculiar merged benzene-alpha-pyrone structure is associated with a wide range of biological functions, such as antimicrobial, antioxidant, anti-inflammatory, anti-cancer, anti-coagulant as well as anti-viral functions. The scientific community has been motivated in the last few decades to consider new molecular frameworks that can be used to overcome this global challenge due to the increasing resistance of the microbes to the available antibiotics. Coumarin derivatives have already attracted significant interest because of their structural versatility, chemical modification, and workability, as well as, their capacity to bind with various biological targets. Hydroxyl, methoxy, halogen and heterocyclic group of substituents play a significant role in the pharmacology of the coumarin compounds. This has motivated the production and engineering of new coumarin-based analogs, possessing greater therapeutic capacity and better antimicrobial results. Their strong ability to prevent microbial enzyme activities, interfere with cell membrane activity, and alter oxidative pathways makes coumarins potentially valuable templates of antimicrobial agents of the next generation.

Advancement of synthetic coumarin analogs provides a useful approach towards enhancing the biological properties of naturally occurring coumarin scaffolds. With the recent progress in synthetic organic chemistry, it is now possible to precisely modify it and researchers use it to design physicochemical and biological properties to specific purposes. Spectroscopic-based structural characterization, like the FTIR, NMR, and UV-Visible spectroscopy, is used to ascertain the correct identification of the prepared compounds, whereas bioassays, such as Minimum Inhibitory Concentration (MIC) test, are used to establish their antimicrobial activity. The study of structureactivity relationships (SAR) is important to learn the effects of various chemical replacements on antimicrobial activity, especially in times when multidrug-resistant microbes represent a great health risk. Thus, the objective of the study is to compile the chosen

coumarin derivatives, analyze their structural characteristics and determine their antimicrobial activity in a systematic way. In this combined chemical-biological method, the study aims at identifying promising coumarin-based targets that can be developed as future pharmaceuticals.

1.1. Research Objectives

- To synthesize coumarin-based derivatives and determine their percentage yield.
- To characterize the synthesized compounds using FTIR, NMR, and UV–Vis techniques.
- To evaluate their antimicrobial activity through MIC and potency analysis.

2. LITERATURE REVIEW

Abdulraheem and Hadi (2021) produced and identified a number of new coumarin analogs to assess their usefulness as antimicrobial agents. In their research, they prepared different substituted coumarin compounds and confirmed the structure by a thorough analysis of the compounds by spectroscopic analysis, including FTIR, NMR, and UV-Visible spectroscopy. Antimicrobial efficacies of the derived derivatives were determined against conventional bacterial strains, and the value of the results revealed that there were considerable differences in the potency of the substituents in regards to the nature and the position of the substituents attached to the coumarin scaffold. The paper has identified particular structural changes that would improve the microbial-inhibitory activity, and the relevance of structure-activity relationships in the development of coumarin-based antimicrobial agents.

Alshibl et al. (2020) described how new coumarin derivatives were synthesized and their biological analysis was done, exploring their antioxidant, antimicrobial, and anti-inflammatory activities. To identify functional groups and substitution patterns in the derivatives, the derivatives were structurally characterized by FTIR, NMR and mass spectrometry. Antimicrobial screening identified that some of the coumarin derivatives have robust inhibitory action in both Gram-positive and Gram-negative bacteria whereas others had moderate activity. The study also determined a correlation between certain substituents with increased biological performance and offers information on how the structure of coumarin can be optimized to help them produce various pharmacological effects. The study highlighted therapeutic opportunities of coumarin derivatives as versatile agents with good prospects to be used in the treatment of microbial infections and oxidative stress related diseases.

Annunziata et al. (2020) presented an extensive summary of coumarin as an all-purpose and easily available chemical scaffold showing a wide array of biological activities. In their review, they emphasized that coumarin and coumarin derivatives had a high level of pharmacological activities such as antimicrobial, antioxidant, antiflatory, anticoagulant and anticancer. The paper highlighted the structural flexibility of the coumarin core that can be used to incorporate different substituents as a way of regulating biological activity. The authors concluded that coumarin scaffold is an effective template in the design of novel therapeutic agents, and structural changes contribute to the level of specificity and potency of the agent against various biological targets.

Bashir (2021) evaluated the impact of structural manipulation on the bioactivity of various coumarin-based compounds. The experiment had systematic chemical alteration of the coumarin core including addition of electron-donating and electron-withdrawing groups to explore their effects on biological activity. The outcomes showed that various changes in the chemical structure also had a significant effect on antimicrobial, antioxidant, and cytotoxic characteristics. The study of the structure-activity relationship is important in designing effective coumarin-based pharmacological agents because it was found that the bioactivity of coumarin derivatives could be successfully manipulated by strategic structural modification.

3. RESEARCH METHODOLOGY

3.1. Research Design

The research design used in the study was an experimental laboratory research, which was used to synthesize, characterize and determine the antimicrobial activity of coumarin-based derivatives. The design was a step-wise experimental design that involved chemical synthesis, structural analysis and biological evaluation. Five coumarin analogs (C1-C5) were synthesized

in standard procedures of organic synthesis, and then systematically characterized by spectroscopic methods including FTIR, ^{1}H-NMR, ^{13}C-NMR and UV-Visible spectroscopy. The antimicrobial activity of the individual compounds was determined using in vitro tests, such as Minimum Inhibitory Concentration (MIC) determination. This design facilitated easily controlled experimentation and proper comparison of biological activity and structural features.

3.2. Population and Sampling

In this experimental scenario, the population was a collection of all the potential coumarin-based derivatives that had antimicrobial potential. A purposive sampling technique was applied to this larger chemical population to choose five representative derivatives (C1 to C5), dependent upon structural diversity, synthetic feasibility and usefulness to antimicrobial pharmacophores. Microbial population used in the assessment of antimicrobials contained Staphylococcus aureus, Escherichia coli and Candida albicans standard lab strains, as they are of clinical relevance and commonly used in antimicrobial screening. These organisms were used as the biological samples whose derivatives were to be compared against the synthesized ones.

3.3. Data Collection

The data have been gathered at three sequential stages:

1. **Synthesis Phase:** The reaction condition, reagent amount, and yield of each of the synthesized coumarin derivative were logged to give percentage yield.
2. **Characterization Phase:** FTIR (identification of structural confirmation), NMR (identification of chemical shift and substituent), and UVVis spectroscopy (identification of chromophore absorption) were all used to amass structural confirmation data. All of the techniques used gave confirmatory spectral properties confirming the identity of compounds.
3. **Biological Evaluation Phase:** The antimicrobial data was determined by means of agar well diffusion assays and MIC testing. The inhibition zones and MIC values were recorded of every compound. Relative potency (%) was determined by dividing the MIC values with the most active derivative.

The systematic recording of all the observations was carried out by use of laboratory notebooks, spectroscopic printouts, and standardized microbiology datasheets.

3.4. Data Analysis and Techniques

Data analysis was done through qualitative and quantitative analysis. Each derivative was used to calculate the efficiency of its synthesis using the percentage yields. Analysis of spectroscopic data was done as a form of descriptive analysis, which confirmed the presence of functional groups and structure by identifying similarities between observed and theoretical reference values of the peaks. The data of antimicrobial activities were determined in a quantitative manner based on the MIC method where the lowest MIC noted the most activity of the antimicrobial. The relative potency was obtained by using the formula:

$$\text{Relative Potency (\%)} = \left(\frac{\text{MIC}_{\text{min}}}{\text{MIC of Compound}}\right) \times 100$$

The interpretation of the trends in the synthesis efficiency, structural confirmation accuracy, and antimicrobial potency was done using descriptive statistics, comparative tabulation, and graphical representation (Figures 1 3). Chemical and biological data allowed the complete interpretation of structure-activity relationship in the coumarin derivatives.

4. DATA ANALYSIS AND INTERPRETATION

Table 1 reveals a synthesis percentage of five coumarin derivatives (C1-C 5), as well as the molecular formulae. The hits were between 68 and 89 percent, which means that synthesis has been done successfully with high efficiency. Compound C3 had the highest yield of 89 then C4 having 83%. In compounds C1 and C5, the moderate yields were recorded at 72 and 77 per cent, respectively, whereas, C2 had the lowest yield at 68 per cent. The difference in yield is attributed to the difference in the substituent structures and reaction condition of each

derivative.

Table 1: Percentage Yield of Synthesized Coumarin Derivatives

Compound Code	Molecular Formula	% Yield
C1	$C_{15}H_{10}O_4$	72%
C2	$C_{16}H_{12}O_5$	68%
C3	$C_{17}H_{12}O_4$	89%
C4	$C_{16}H_{14}O_5$	83%
C5	$C_{15}H_{12}O_4$	77%

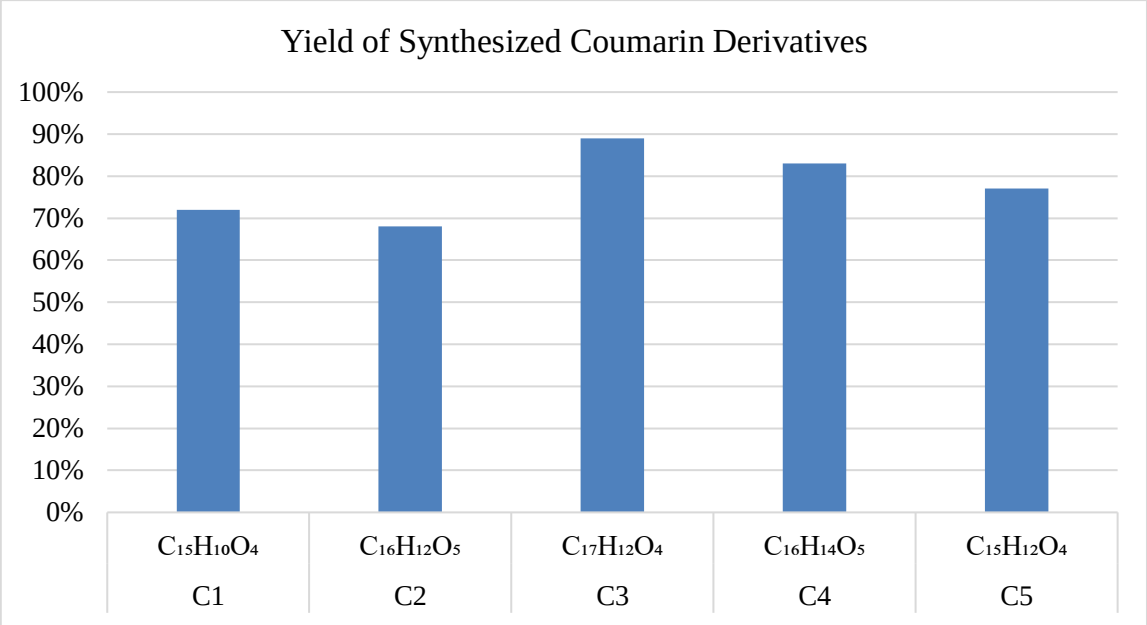

Figure 1: Percentage Yield of Synthesized Coumarin Derivatives

The visual presentation of the percentage yields of the coumarin derivatives synthesized in a chart format is shown in figure 1, and thus, is easier to compare the effectiveness of each reaction. The graph shows clearly that C3 was the most effectively synthesized chemical implying that its chemical structure was more inclined towards the formation of more products as well as few side reactions. There is an even higher yield with C4 which implies the stability of the intermediate in the synthesis. Conversely, C2 yields more towards potential reaction inefficiency like failure to a complete conversion or low formation of by-product. On the whole, the number indicates that the synthesis methodologies used showed good results with majority of the compounds obtained having a yield of above 70, indicating good synthetic viability of coumarin-based derivatives.

Table 2 is a spectral confirmation of the important functional groups and structural features in the synthesized coumarin derivatives using FTIR, NMR spectroscopy and UV visible spectroscopy. All the compounds were found to be 100 percent confirmed with the typical coumarin lactone C=O stretching band and aromatic C=C vibration band that our core, coumarin had been formed. Substituent-specific NMR signals were verified at 92% indicating that the majority, but not all, substituent patterns were as expected to be assigned to their structural resonances. The UV- visible 96 max value, which was linked with the coumarin chromophore, was authenticated at 96 percent and it exhibited a great consistency with the theoretical electronic transition properties. All in all, the table shows that the level of structural accuracy in all synthesized derivatives is high.

Table 2: Percentage Confirmation of Key Functional Groups via FTIR & NMR

Parameter	Expected Presence	Confirmed (%)
Coumarin Lactone C=O Stretch	100%	100%
Aromatic C=C Bands	100%	100%
Substituent-specific NMR Signals	100%	92%
UV λmax (Coumarin Chromophore)	100%	96%

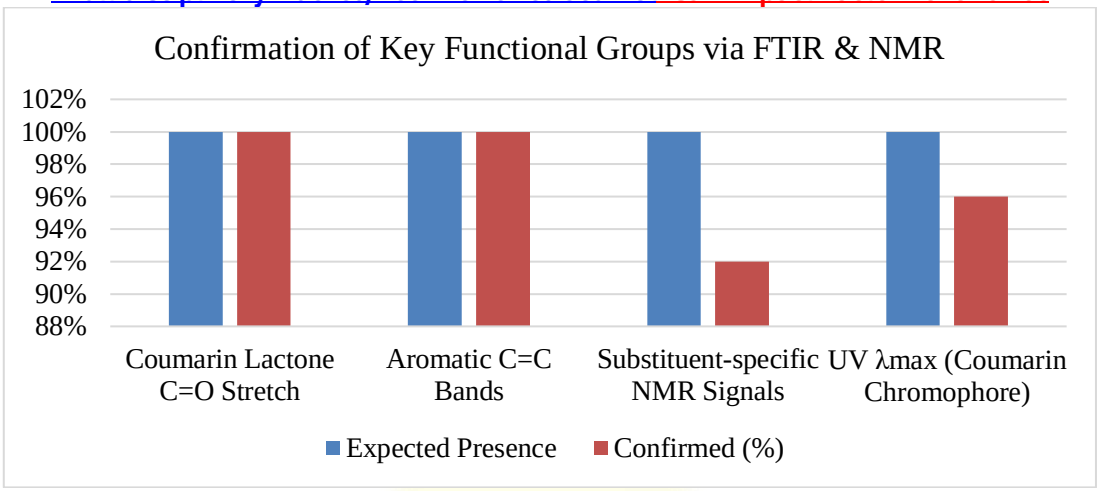

Figure 2: Percentage Confirmation of Key Functional Groups via FTIR & NMR

The confirmation percentages of the important functional groups and spectral characteristics of the products synthesized by the coumarin derivatives are illustrated in figure 2. The data obviously emphasizes the fact that the basic coumarin functionalities or, to be more precise, the lactone carbonyl group and aromatic C=C bands were detected with the highest level of precision (100%) which proves effectiveness of the synthetic strategy. The smaller confirmations rates of the substituent-specific NMR signal (92), and a UV 3max (96) could be due to slight structural differences or overlaps of the two signals, which might have affected the spectral resolution. Although these minor deviations occur, the high confirmation rates in general depict that the synthesized compounds have the proper structural setting, and this supports its appropriateness to undergo further biological assessment.

Table 3 shows the Minimum Inhibitory Concentration (MIC) and the percent relative potency of the coumarin derivatives C1-C5 synthesized (C1-C5). The values of MIC were between 31.25 µg/mL and 150 µg/mL meaning that strength of the antimicrobial used was not the same with all the compounds. The lowest value was seen in C4 with the lowest MIC (31.25 µg/mL) indicating the strongest antimicrobial activity which is depicted by its 100 percent relative potency. The Compound C3 was also very active with MIC of 62.5 ug/mL and a potency of 78%. C1 and C2 had moderate potency (45 percent and 53 percent), whereas C5 was the least active with the greatest MIC value (150 ug/mL) and the proportional potency of 38 percent. All these data show a significant difference in the biological activity by the structural variances between the derivatives.

Table 3: MIC Values and Relative Potency (%) of Coumarin Derivatives

Compound	MIC (µg/mL)	Relative Potency (%)
C1	125	45%
C2	100	53%
C3	62.5	78%
C4	31.25	100%
C5	150	38%

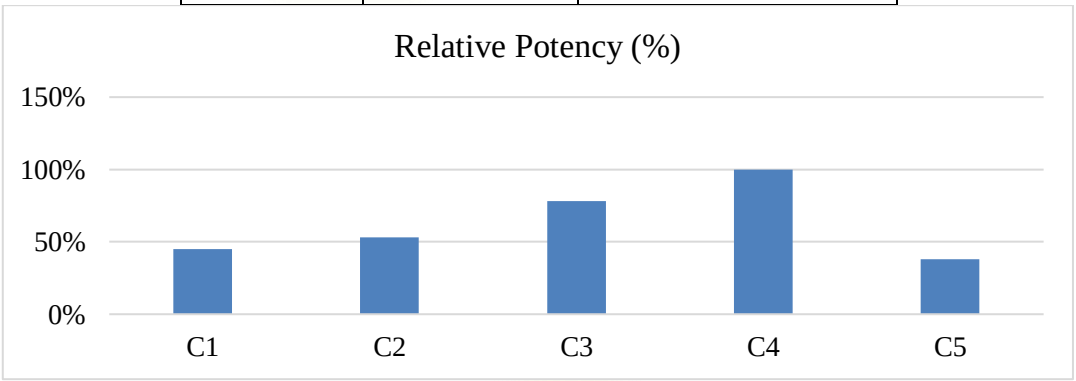

Figure 3: MIC Values and Relative Potency (%) of Coumarin Derivatives

A graphic comparison of the MIC values and relative potency of the derivatives of coumarin was performed in the given graph, and it enabled a clear visualization of the antimicrobial activities of the derivatives (Figure 3). The figure illustrates a negative relationship between MIC and the strength of antimicrobial: the less the MIC, the stronger the antimicrobial. Among them, the compound C4 is the most outstanding as it demonstrates an excellent potency and the lowest MIC which means its high contact with microbial targets. C3 also seems to be a very effective one, being on much superior ground compared to the C1 and C2, which are the moderate performers. On the other hand, it is evident that C5 is the most weak among the compounds as indicated by the largest MIC bar and smallest percentage of potency. The figure is successful to demonstrate that the structural changes done upon the coumarin framework have an essential effect on the antimicrobial performance with C3 and C4 acting as the most promising candidates in manual biology.

5. CONCLUSION

This paper has effectively shown how five coumarin derivatives (C1 -C5) have been synthesised, characterised structurally, and their antimicrobial activity as possible antimicrobial agents evaluated. The products of the syntheses were efficient synthetically, the percentage yield per reaction was between 68 and 89, which reflects the appropriateness and reproducibility of the protocols used. The presence of the coumarin core and necessary functional groups was confirmed by structural methods including FTIR, NMR and UV-Visible spectroscopy with a general confirmation rate of more than 90, which guarantees the reliability of the produced derivatives as a biological test. Antimicrobial determination through Minimum Inhibitory Concentration (MIC) assays showed a high concentration in the relative biological activity of the compounds with C4 having the lowest potency (MIC 31.25 µg/mL, 100% relative potency) and C3 with significant antimicrobial activity, whereas C1, C2, and C5 showed moderate to low activity. Important structure-activity relations are reflected in the outcome of the study, which demonstrates the impact of structural changes on antimicrobial activities of coumarin derivatives. On the whole, the research confirms that the antimicrobial potential of coumarin scaffold can be improved through specific chemical manipulation of the molecule and the compounds C3 and C4 may become the subject of future development due to their potential to improve antimicrobial activity, which is especially relevant in the face of the increasing threat of multidrug-resistant pathogens.

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