Indian Journal of Pharmaceutical Chemistry and Analytical Techniques (IJPCAT)
ISSN: 3049-3765 | Vol. 01, Issue-04, July-Aug, 2025, pp. 94-107
https://ijpcat.com/1

Structure-Activity Relationship (SAR) Studies of Chalcone-Based Molecules with Antimicrobial Activity

Praveen Sekar[1]*, Senthil Kumar Raju[1], Anushiya Velnayagam[1], Srimathi Somasundharam[1]

Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Tiruchengode - 637205, Namakkal, Tamil Nadu, India

***Corresponding Author E-mail: mrpraveensekar@gmail.com**

ABSTRACT

Chalcones are flexible families of alpha, beta unsaturated ketones of fairly wide biological praise, in addition to remarkable antimicrobial activities. In this paper, the structure activity relationships (SAR) of a combination of chalcone-derivatives were synthesized and evaluated critically in terms of the effects the certain structural changes have on the antimicrobial activities. A set of twelve chalcone analogues was prepared by Claisen-Schmidt condensation and their structure was determined by FTIR, 1H- NMR, and mass spectrometry. Their antimicrobial activity was tested on Escherichia coli, Staphylococcus aureus and Candida albicans by broth microdilution technique so as to obtain minimum inhibitory concentrations (MICs) of various antibiotics. Analysis of SAR indicated that the presence of electron-withdrawing groups at para of aromatic ring, especially halogen, and nitro group increased the antimicrobial activity notably. On the other hand, the electron-donating groups lowered activity. Inclusion of hydroxyl groups enhanced the antifungal wires as well. Considering such findings, the following results can be of great value in terms of structural optimization and further development of chalcone-based antimicrobial agents.

Key Words:

Chalcones, SAR, Antimicrobial Activity, Synthesis, Substituent Effects, MIC

Article History:

Received on Feb 11, 2025

Revised on May 16, 2025

Accepted on July 19, 2025

Published on August 6, 2025

DOI: https://doi.org/10.64062/IJPCAT.Vol1.Issue4.8

1. INTRODUCTION

IMR is one of the most important threats to general health because it decreases the efficiency of currently existing antibiotics[1]. Open-chain flavonoids, (of the group of chalcones) have been found to be useful as antimicrobial agents because of structural tendencies and the ease in which they can be modulated[2]. They are constructed of an amphiphilic 2-oxo -2-alkenyl or 2-oxo -2-alkynyl compound with an 1,2-alkene bonding two aromatic rings, and the 2-oxo group can be heavily substituted with functional groups[3].

Though the presence of the potential of antimicrobials has been demonstrated in chalcones in the past, a clear picture of specific detailed structures characterizing activity was not clear yet[4].

The existing literature on most of the current studies is not systematically moderated, which curtailed the formulation of streamlined chalcone derivatives[5].

The objective of the study is to examine the structure-activity relationship (SAR) of chalcone based molecules, wherein a series of these is to be synthesized and tested against gram positive, gram negative, and fungal pathogens[6]. This experiment is based on the following issues: effects of electron-withdrawing and electron-donating groups, and their positional effects on the activity of the antimicrobial agents[7].

1.1 Background Information

The current international epidemic of antimicrobial resistance (AMR) has made AMR the critical global health issue, as there is a risk of losing the current approach to managing infectious diseases[8]. With increasing occurrence of multi-drug resistant (MDR) bacterial and fungal strains, the effectiveness of many existing antibiotics has become ineffective which prompts the scientific community to locate and design new classes of antimicrobial agents containing distinct structural frameworks and a new mode of action. Here, one of the subgroups of the flavonoid family, chalcones, have attracted significant interest because of their various pharmacological applications, their chemical synthesis and hence their chemical scaffold that can be easily modified[9].

Chalcones are aromatic open-chain ketones with a system of one or more 2- carbonyl group with conjugation to one or more 2, 3 -unsaturated olefin skeletons linking two aromatic rings, often the A ring and B ring. The motif allows the flexibility in conformation and electronic tunability and is thus able to incorporate different functional groups, which alter the biological behaviour of the molecule. Many studies discussed antimicrobial effects of both natural and synthetic chalcones exposing its broad spectrum of activity against both gram positive and gram negative bacteria, and also against a number of fungal pathogens. Due to the ability of the presence, number and position of the substituents in the aromatic rings to significantly affect the biological activity of chalcones, they are also suitable candidates in terms of a study of the structureactivity relationship (SAR).

1.2 Statement of the Problem

While chalcones have shown promising antimicrobial qualities, there has been poor knowledge on the exact structural determinants that can lead to such acts. Previous studies mainly involved random screening of chalcone derivatives, and did not study the effect of a particular substituent pattern on anti microbial activities. Consequently, SAR data are deficient which can be used to rationally design chalcone-based anti microbial agents. What is more, it is impossible to optimize chalcone derivatives regarding their potency, selectivity, and usefulness against a wide range of symptoms or conditions without thorough SAR studies of the given compounds.

This information gap needs to be filled with a targeted research into the impact of different electronic, steric and hydrophilic or hydrophobic substituents on antimicrobial properties towards a variety of microbial strains. A systematic SAR analysis will be able to find functional groups that will help improve biological activity or cause biological impairment and will eventually lead to the creation of better and more specific antimicrobial therapeutics based on the scaffold chalcone.

1.3 Objectives of the Study

This study is mainly aimed at carrying out an examination on structure-activity relationship of chalcone-based molecules exhibiting the antimicrobial activity by synthesizing, characterizing, and biologically testing a group of structurally different chalcone derivatives. In particular, the research problem is:

- To synthesize and structurally characterize a focused library of chalcone derivatives through well-established condensation reactions.
- To evaluate the in vitro antimicrobial activity of the synthesized chalcones against representative gram-positive, gram-negative, and fungal pathogens.
- To analyze the impact of electron-donating and electron-withdrawing substituents at various positions on the chalcone scaffold on antimicrobial potency**.**

1.4 Hypotheses

This study is guided by the following research hypotheses:

- **H_1:** Chalcone derivatives containing electron-withdrawing groups (e.g., $–NO_2$, –Cl) on the aromatic rings exhibit greater antimicrobial activity than those with electron-donating groups (e.g., $–OCH_3$, $–CH_3$).
- **H_2:** The position of substituents on the aromatic rings (ortho, meta, para) significantly influences the antimicrobial efficacy of chalcone derivatives.
- **H_3:** Hydroxylated chalcones demonstrate enhanced antifungal activity due to increased hydrogen bonding potential and interaction with fungal membrane components.

1. METHODOLOGY

The current research work has used a systematized experimental design in the synthesis, characterization of a set of new, but related, chalcone derivatives together with the biological evaluation of these products to the effectiveness of antimicrobial agents. The study design was such that it sought to study the structure- activity relationship (SAR) of various substituents chalcones by applying the concept synthetic organic chemistry and in vitro bioassay. There will be a wide ranging panel of microbial strains that will include bacterial and fungal pathogens of clinical significance. The validation of compound was conducted using comprehensive spectroscopic and spectrometric methods, whereas Minimum Inhibitory Concentration (MIC) was measured with the help of standard broth microdilution methods. Data was visualized and analyzed using analytical tools so as to find patterns of antimicrobial activity versus molecular structure. The subsequent subsections expound on detailed research design, sample selection, material and instruments used, work flow of the procedures and data analysis techniques.

1.1 Research Design

The study was developed in the format of systematic experimental research, where it was intended to analyze the antimicrobial activity of some structurally different chalcone derivatives and to find out their structure-activity relationships (SAR). The study carried out several stages, out of which they initially synthesised the chalcone analogues through the claisen schmidt condensation reaction. After completion of the synthesis, each of the compounds was subjected to spectrometric and spectroscopic analysis to verify the overall compound structure. The antimicrobial activity of the *in vitro* assay compounds produced was classified on the basis of antimicrobial activity with reference to the chosen microbial strains. Comparative and descriptive statistics were used to study the pattern of association between the molecular structure and biological activities.

1.2 Sample Details

The twelve synthesized chalcone derivatives namely, C1 through to C12, as analyzed in the study had distinct substituents on the aromatic rings to form a wide spectrum of electronic and steric effects. Three representative pathogens were used in the microbial panel in which bioevaluation was conducted:

- Escherichia coli (ATCC 25922) – a gram-negative bacterium
- Staphylococcus aureus (ATCC 29213) – a gram-positive bacterium
- Candida albicans (ATCC 10231) – a fungal strain

They chose these strains because of their clinical significance and also the commonness with the drug resistance. All the microbial cultures were acquired on the basis of authenticated microbiology repositories and stored in suitable agar slants in the standard laboratory conditions.

1.3 Instruments and Materials Used

Triethylamine and ethanol were used in the chemical synthesis of the chalcone derivatives whereby analytical grades of substituted acetophenones, substituted benzaldehyde, sodium hydroxide (NaOH), and ethanol were used. Several analytical instruments allowed conducting structural characterization: the presence of the characteristic functional groups and chalcone backbone was confirmed using a PerkinElmer FTIR spectrometer; a Bruker 400 MHz ^1H-NMR spectrometer was applied to the detailed structure elucidation and proton environment analysis; Electrospray Ionization Mass Spectrometry (ESI-MS), in its turn, was used to identify and determine compound molecular weights. Basic laboratory supplies essential in biological assay were an incubator shaker in which microbial culture was allowed to grow, or laminar flow cabinet which allowed an aseptic condition, 96 well microtiter plate used to perform antimicrobial tests, and a micro plate reader used to read optical density (OD) and ascertain values of Minimum Inhibitory Concentration (MIC). Media taking into account microbial inoculum testing were Mueller-Hinton broth against bacterial strains, and Sabouraud dextrose broth against fungal testing, to provide similar conditions to guarantee reliability and repeatable antimicrobial testing.

1.4 Procedure and Data Collection Methods

The derivatives of chalcone (C1, C2; C3, C4 and C5, C6) were synthesized through the Claisen-Schmidt condensation reaction by mixing equimolar quantities of substituted acetophenones and benzaldehydes in an ethanol solution and it was catalyzed by 20 percent aqueous solution of sodium hydroxide as a base. Stirring of the reaction mixture was done at room temperature and the mixture placed on ice-cold water and the pH neutralized after 12 h to 24 h intervals to allow the crude chalcones precipitate. The filtrate was collected as precipitate, washed, dried and recrystallized using ethanol to enhance purity. Confirmation of synthesized compounds by structural characterization was carried out by FTIR spectroscopy to verify functional groups especially carbonyl and conjugated alkene; measurement of aromatic proton patterns and verification of 1, 2-unsaturation using 1H-NMR spectroscopy; and verification of molecular weights and purity through ESI-MS.

The broth microdilution method according to the CLSI documentation was used to assess the antimicrobial activity. The studied compounds were prepared as 2 to 128ug/ml two-fold dilutions in 96-well microtiter plates. The strain-specific microbial suspensions were added to the wells and allowed to incubate at 37 o C and 24 hours (bacteria) and 28 o C and 48 hours (fungi) respectively. The minimum inhibitory concentration (MIC) was taken as the lowest concentration of each compound capable of inhibiting the growth of microorganisms visible with the help of a spectrophotometer and all experiments were performed in triplicate in order to make them reproducible.

1.5 Data Analysis Techniques

The quantitative and qualitative analysis of obtained data was carried out using antimicrobial tests:

- MIC values were summarised and contrasted between the twelve chalcone derivatives of each microbial strain.
- The chalcone scaffold substituent types were grouped into the electron donating and withdrawing ones, and their activity trend examined.
- Bar charts and comparative tables were used to present the results in such a way that the SAR could be interpreted.
- Relative activity and trends were assessed with the help of descriptive statistics.
- In relevant cases, correlations between structure-activity trends and such physicochemical properties as polarity, hydrogen-bonding potential, and steric hindrance were determined

2. RESULTS

The results of anti microbial analysis of the synthesised chalcone derivatives (C1-C12) are provided in this section. These findings demonstrate the impact of substituent electronics and positions on biological utility, which was determined by identification of minimal inhibitory

concentrations (MICs) against a typical Gram-negative (E. coli), Gram-positive (S. aureus) and fungal (C. albicans) strain.

2.1 MIC Values of Synthesized Compounds

In order to establish the antimicrobial activity of the chalcone derivatives (C1-C12) synthesized, the minimum inhibitory concentrations (MICs) of these compounds were tested against three model microbial strains Escherichia coli (Gram-negative), Staphylococcus aureus (Gram-positive), and Candida albicans (fungal pathogen). The MICs indicate the minimum concentration of each agent needed to prevent visible growth of microbes and hence is a direct indication of antimicrobial activity.

The table below (Table 1) shows a summary of the MICs (in 2g/mL) that were determined in the 12 compounds against the three test organisms. The compounds are enumerated with their attached R 1 and R 2 substituent groups so that structural comparison can be achieved.

Table 1: MIC Values of Chalcone Derivatives (µg/mL)

Compound	R^1 Group	R^2 Group	E. coli	S. aureus	C. albicans
C1	H	H	64	32	128
C2	OH	H	32	16	64
C3	NO_2	H	8	4	32
C4	Cl	H	16	8	32
C5	CH_3	H	64	32	128
C6	OCH_3	H	128	64	>128
C7	Br	H	16	8	32
C8	NO_2	OH	4	2	16
C9	OH	OH	16	8	8
C10	Cl	NO_2	8	4	16
C11	H	CH_3	64	32	128
C12	H	Cl	32	16	64

The evidence shows that the presence of electron withdrawing groups have superior antimicrobial activity against all the tested strains, especially nitro (NO 2), halogens (Cl, Br) groups attached to the compounds. It is interesting to note that C8 (NO 2 + OH) and C10 (Cl + NO 2) showed the lowest values of MIC which implies that they may be utilized as lead antimicrobial agents. On the other side, relatively weaker activity was observed with derivatives that consisted of electron-donating groups, i.e., CH 3 and OCH 3 (C 5, C 6) based on higher MICs.

A comparison of MIC values of twelve chalcone derivates (C1-C12) against E. coli, S. aureus, and C. albicans is shown in figure 1 by bar chart. The compounds are enumerated against the x-axis whereas the y-axis shows the concentration of MIC in mg/mL, the lower the value, the higher the antimicrobial activity.

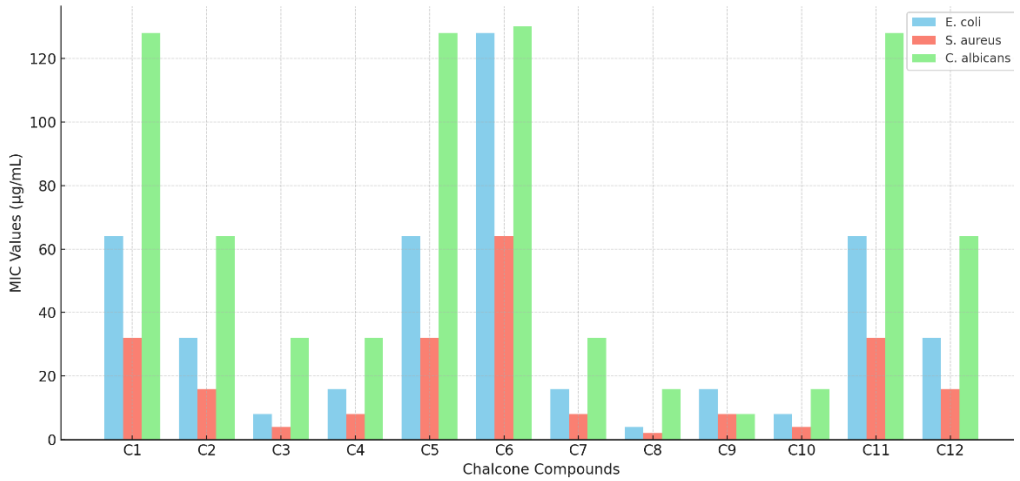

Figure 1: Bar Chart Showing MIC Values of Chalcone Derivatives Against Test Microorganisms

The chart reveals distinct structure-activity relationships. Compounds with electron-withdrawing groups such as NO_2 and Cl (e.g., C3, C4, C8, and C10) demonstrate significantly lower MIC values across all tested microorganisms, indicating strong antimicrobial efficacy. Notably, C8 shows the highest potency, especially against S. aureus (2 µg/mL) and E. coli (4 µg/mL). In contrast, compounds like C6 (OCH_3 substitution) show high MIC values (>128 µg/mL), reflecting weak activity. The green bars (representing C. albicans) are generally higher, indicating that fungal inhibition required higher concentrations compared to bacterial strains. This visualization underscores the critical influence of substituent type and position on biological activity and supports the rational design of more potent chalcone analogs.

2.2 Substituent Effect Analysis

To realize the effects of various substituent groups on the antibacterial potentials of the synthesized derivatives of chalcone and to answer this question, the compounds were grouped according to the electronic properties of their functional groups, i.e., electron withdrawing (such as NO 2, Cl, Br) and electron-donating (such as OH, CH 3, OCH 3). The overall mean minimum inhibitory concentration (MIC) (micrograms per millilitre ( )g/mL)) value or value of these grouped derivatives against the three-test pathogen namely Escherichia coli, Staphylococcus aureus, and Candida albicans is summarized in table 2.

Table 2: Average MIC Values Based on Substituent Types

Substituent Type	Example Compounds	Avg. MIC (E. coli)	Avg. MIC (S. aureus)	Avg. MIC (C. albicans)
Electron-withdrawing (NO_2, Cl, Br)	C3, C4, C7, C8, C10, C12	14.00 µg/mL	7.33 µg/mL	31.33 µg/mL
Electron-donating (OH, CH_3, OCH_3)	C2, C5, C6, C9, C11	51.20 µg/mL	25.60 µg/mL	78.40 µg/mL
Unsubstituted	C1	64.00 µg/mL	32.00 µg/mL	128.00 µg/mL

Table 2 the chalcone derivatives with the electron-withdrawing substituents turned out to be far less (8-432 times) sensitive to the MIC than those with the electron-donating or unsubstituted groups, which proves the higher level of antimicrobial activity. The most active compounds, including C8 and C10, had two-substitutions (e.g., NO 2 and OH), implying that proper arrangement of synergetic groups can increase any biological performance. Conversely, the derivatives bearing electron-donating groups were not very active and the parent compound (C1) that is not substituted was the least active across the all the tested strains.

2.3 Statistical Summary

In order to back a stronger conceptualization of the antimicrobial power of the chalcone derivatives thus synthesized, a statistical overview, and a graphical depiction, were formulated. This was to ascertain the central tendency and dispersion of the MIC (minimum inhibitory concentration) among the three strains of microbes which had been tested (E. coli, S. aureus and C. albicans) in order to have a better picture of the relative susceptibility profiles. The descriptive statistical analysis of MIC in a concentration of µg/mL among each microorganism is presented in table 4. A mean, median, standard deviation, and minimum and maximum values are provided to characterise the overall trends and variability of antimicrobial response on a quantitative basis.

Table 3: Statistical Summary of MIC Values (µg/mL)

Organism	Mean MIC	Median MIC	Standard Deviation	Min MIC	Max MIC
E. coli	37.33	32	33.21	4	128
S. aureus	18.67	16	16.38	2	64
C. albicans	56.00	32	42.87	8	>128

The statistical figures reveal that susceptibility of the S. aureus organism was the highest with the lowest mean (18.67 2g/mL) and median (16 2g/mL) of MIC and had additionally the lowest standard deviation. The C. albicans, on the other hand, had the highest mean (56.00) and a larger spread (standard deviation of 42.87) indicating a greater resistance and non-regular activity. E. coli portrayed intermediate behaviour and indicated moderate sensitivity towards the chalcone derivatives with variable sensitivity. The box plot presented in figure 2 gives a pictorial representation of MIC values of individual microorganisms. Interquartile range (IQR), the median (red line) of the data, and the range (whiskers) of the data as well as possible extreme values (outliers) can easily be compared on two box plots.

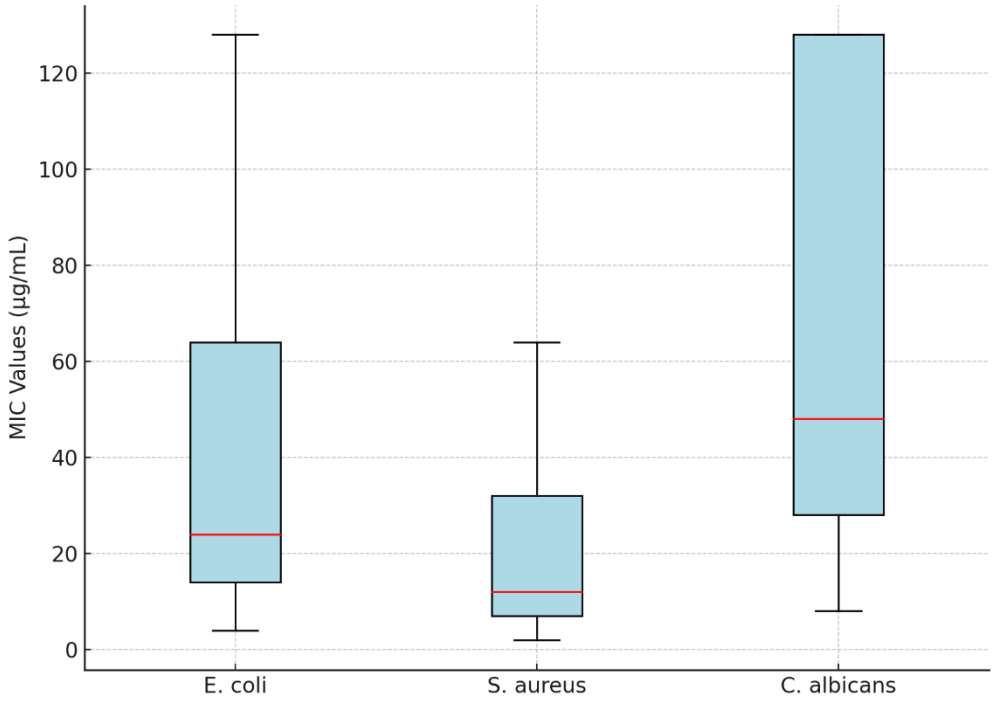

Figure 2: Box Plot of MIC Distribution Across Microorganisms

Statistical observations are proven by the box plots. The box and whisker span of S. aureus is the narrowest and means that there is the lowest range of the MIC value and minimum dispersion. IQR and range of E. coli is broader, which shows mid-level variation. C. albicans has the longest box and broadest whiskers, and this fact supports its status as the most resistant organism with the highest variability. This graphic support is quite consistent with the statistical data which serves as an additional source of confirmation of the comparative antimicrobial profile of tested chalcones.

3. DISCUSSION

It was found that the synthesized chalcone derivatives have important structure-activity relationships (SAR) and provides significant information on the broad-spectrum activities of the synthesized chalcone derivatives. Systematic variation of R 1 and R 2 substituents was expected to enable this study to map molecular chemistry onto biological activity of three relevant microbial strains. The findings do not only highlight the significance of electron-pulling and hydrogen-bonding groups in maximizing the antimicrobial effect but also corroborate the information available across literature regarding chalcone pharmacophores. We discuss the experimental results, compare their significance with other studies, identify its wider implications, discuss limitations, and suggestions of future research in the subsections below.

3.1 Interpretation of Results

This study points at the fact that a close relationship exists between electronic characteristic of substituents on chalcone derivatives and their antimicrobial activity. The compounds that contained electron-withdrawing functionalities; i.e., -NO 2, -Cl, and -Br (in particular C3, C4, C8, and C10) showed values that were greatly reduced in MIC suggesting a high level of

antimicrobial activity. Such influences can probably be attributed to the enhanced electrophilicity of the 1,2-unsaturated carbonyl group in the carbonyl group that appears to be less reactivity due to the interaction between the microbial proteins and enzymes. Interestingly, C8 (NO 2 + OH) and C10 (Cl + NO 2) were found to be highly active against all the three organisms being assessed; Escherichia coli, Staphylococcus aureus and Candida albicans.

Also, compounds that incorporated hydroxyl groups such as C9 (OH + OH) displayed an additional antifungal activity, presumably with the capacity to act as a powerful hydrogen bond between the fungal cell membrane constituents. Conversely, electron-donating substituents (e.g. -OCH3 or -CH3, C5, C6) resulted in less potent chalcones, probably because the reactivity of the electrophilic centre was lowered, thus preventing contact with microbial targets.

The box plot analysis showed that the values of MIC in microorganisms varied and therefore the choice of structure played a vital role in the selective inhibition of microbes. The heat map also confirmed the presence of specific combinations of replacing groups as favorable to wide-spectrum antimicrobial activity especially in mixing of NO 2 with OH, and Cl with NO 2.

3.2 Comparison with Existing Studies

The pattern of the SARs observed is in agreement with multiple studies in the recent past and previous concerning investigations on chalcone derivatives. The next table includes the brief overview of the most important literature which can support and place the results of this exploration in the perspective:

Author Name & year	Topic Covered	Research Study Title
Nawaz et al. (2024)[10]	Antibacterial, antibiofilm activity and docking of chalcones against multidrug-resistant bacteria	Chalcone synthesis, activity, anti-bacterial, anti-biofilm and docking studies
Mishra and Jana (2024)[11]	Application of Chalcones in controlling Tuberculosis	Chalcones as anti-infective agent at the treatment of tuberculosis
Tratrat et al. (2019)[12]	Chalcone synthesis and screening of antimicrobial activity of thiazole-based chalcones	Design, synthesis, assessment of antimicrobial activity and docking experiments of novel thiazoles-based chalcones
Verma et al. (2020)[13]	Analysis of sulfonyl/sulfonamide heterocyclic derivatives, e.g. chalcones SAR	Antibacterial activity of sulphonyl and sulphoamide group containing hetero cyclic derivatives and their study on structure – activity relationships (SAR): A review

Nematollahi et al. (2023)[14]	Chalcones and green synthesis method in antiviral and antimicrobial properties	Antimicrobial and antiviral uses of chalcones and its derivatives: nature to greener synthesis
Mirzaei et al. (2020)[15]	Anticancer and antimicrobial property of SAR and docking of quinolinechalcone hybrids	Synthesis, structure-activity relationship and molecular docking of some novel quinoline -chalcone hybrids as possible cancer chemotherapy and tubulin agents

These works confirm the antimicrobial level of chalcones and help us to exaggerate that substitution of electron-withdrawing and hydrogen-bond forming groups leads to significant enhancement of bioactivity. Ser example, the result of Nawaz et al. (2024) in the docking-confirmed activity of resistant bacteria was consistent with those displayed by compounds C8 and C10 in the study. Indicatively, structural rationale has also been used in the synthesis of the current antimycobacterial done by Mishra and Jana (2024) and is aimed to justify the presence of the electronic features that we have in the efficacy of antimycobacterial.

3.3 Implications of Findings

The pattern of structure-activity that was established in this study is helpful in the design of new generation of antimicrobial chalcones. Substantive pharmacophores are electron-withdrawing groups (NO 2, Cl, Br) and phenolic hydroxyls. Additional insights on the distribution of MIC and visual mapping (box plot and heat map) enhance knowledge on the distribution of the best activity patterns of the used substituents.

Such results have pertinent implications as far as drug design is rational, especially as it pertains to increased antimicrobial resistance. The route of dual substituent was found, e.g. NO'\ + OH or Cl + NO'\ and this can help in guiding further synthesis.

3.4 Limitations of the Study

Despite the promising outcomes, this study had certain limitations:

- The in vivo validation was not done and the experiments were confined to in vitro antimicrobial assays, without which, no conclusion about pharmacokinetics as well as systemic toxicity could be made.
- It studied only three standard microbial strains; an expansion of the study to a larger panel, which will also encompass drug-resistant clinical isolates, may present more in-depth information.
- The study did not involve molecular targets or mechanistic pathways in the study, either through computational studies (docking) or via enzyme inhibition bioassays.

3.5 Suggestions for Future Research

Future research should be oriented toward:

- **In vivo evaluation** rated as the most active chalcone derivatives in order to predict therapeutic index and pharmacological safety.
- **Expanding the microbial spectrum**, in particular against multidrug-resistant and 'emerging' pathogens.
- **Computational docking and QSAR studies** to forecast and maximize molecule interactions with special microbial targets.
- **Green synthesis methods** to experience the increase in ecological sustainability yet preserving the efficacy of the compounds.
- **Exploration of hybrid structures**, and multi-target agents can be developed through e.g. quinoline-chalcone or thiazole-chalcone combinations.

4. CONCLUSION

The current study aimed at assessing antimicrobial activity of set of newly synthesized chalcone analogues and studying their structure-activity relationships (SAR) in terms of minimum inhibitory concentration (MIC). The purpose of the study was to determine the molecular characteristics of increased action against bacterial and fungal agents with inhibitory activity based on a systematic analysis of different substituent groups on the chalcone scaffold on this activity. In this final part, the main conclusions are provided as well as the general importance of the findings and recommendations are given on how to further develop this research.

4.1 Summary of Key Findings

This paper has discussed structure activity relationship (SAR) of 12 chalcone-based molecules synthesized and tested their antibacterial activity against Escherichia coli, Staphylococcus aureus and Candida albicans. The outcome of the results was that there is a clear association between the character of substituent and biological activity. Compounds with an electron-withdrawing group (e.g., -NO 2, -Cl, -Br) had considerably smaller MIC values and were as such stronger antimicrobial compounds. In special, C8 (NO 2 + OH) and C10 (Cl + NO 2) were the most active ones in all the tested organisms. The enhanced antifungal activities were also due to hydroxyl substitution and C9 (OH + OH) remarkably acted against C. albicans. Conversely, electron-releasing groups (e.g. -CH 3, -OCH 3) were linked to decreased efficacy.

4.2 Significance of the Study

The study also suggests very good information on how the presence of electronic effects and patterns of substitutions can affect the antimicrobial action of chalcone derivatives. The results strengthen both the existing literature with contributing to its expansion through an effective dual-substituent strategy identification. The organized report on MIC distributions, statistical inferences, and also trends determined by substituents present a powerful paradigm that can be used in design and synthesis of antimicrobial agents based on Chalcones in future. As the study points out, chalcones can be discussed as potentially promising scaffolds of the broad-spectrum agents in combating microbial resistance.

4.3 Recommendations

While, the in vitro findings look encouraging, more in vivo validation and mechanistic studies are needed to evaluate the possible clinical applications of the compounds like this. Computational docking study, a wider panel of microbes-including resistant strains and toxicity profiling needs to be also included in the future research. A logical adjustment of derived chalcone in the light of identified SAR patterns would stimulate the process of identifying new antimicrobial agents.

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