Poonam Kumari, 2026, 14:2
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Synthesis, Structural Characterization, and Biological Evaluation of Salicyaldehyde-Derived Fluorinated Chalcones with Enhanced Antimicrobial Activity

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Abstract- A series of novel fluorinated chalcone analogues incorporating a hydroxybenzaldehyde framework was successfully synthesized and systematically investigated. The synthetic approach involved a base-catalyzed Claisen–Schmidt condensation between various hydroxy-substituted benzaldehydes and fluorinated acetophenone derivatives. The structures of the synthesized compounds were unequivocally established using detailed spectroscopic techniques, including ^1H NMR, ^13C NMR, infrared spectroscopy, and mass spectrometry. The antimicrobial activity of the prepared chalcone derivatives was evaluated against a panel of clinically relevant bacterial and fungal strains, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. Several compounds demonstrated significant antimicrobial potency, with minimum inhibitory concentration (MIC) values ranging from 5 to 25 µg/mL. Structure–activity relationship (SAR) studies revealed that the antimicrobial efficacy is strongly influenced by the number and positional arrangement of hydroxyl groups, as well as the electronic effects imparted by fluorine substitution within the chalcone scaffold. In conclusion, the study highlights the potential of fluorinated hydroxychalcone derivatives as promising antimicrobial candidates and provides important insights for the rational design and development of more potent next-generation antimicrobial agents.

Keywords: Salicyldehyde derivatives, Chalcones, Antibacterial activity, Antifungal Activities.

I. INTRODUCTION

Chalcones represent a significant class of both naturally occurring and synthetically derived flavonoid analogues and are widely recognized as versatile molecular scaffolds associated with a diverse range of biological activities. Structurally, these compounds are characterized by an open-chain α,β-unsaturated carbonyl system, wherein two aromatic rings (commonly denoted as rings A and B) are linked through a three-carbon enone bridge. This structural motif renders chalcones important biosynthetic intermediates in the formation of flavonoids and isoflavonoids [1,2]. Owing to their broad pharmacological profile, encompassing anticancer, anti-inflammatory, antioxidant, and notably antimicrobial properties, chalcones have garnered substantial attention for structural modification and optimization [3–5]. Variations in substituents on the aromatic rings have been shown to markedly influence biological activity, thereby enabling the modulation of potency and selectivity in drug design strategies [6,7].

Among the various modification approaches, the incorporation of fluorine atoms has emerged as a particularly effective strategy for enhancing biological performance. Fluorine substitution often imparts advantageous physicochemical properties, including increased lipophilicity, improved metabolic stability, and enhanced interactions with biological targets, largely due to its high electronegativity and small atomic radius [8,9]. As a result, fluorinated chalcone derivatives frequently demonstrate superior biological activities, especially in terms of antimicrobial efficacy, compared to their non-fluorinated counterparts [10,11].

In addition, the introduction of hydroxyl groups at specific positions on the aromatic rings plays a crucial role in modulating biological activity, primarily through the establishment of hydrogen-bonding interactions with target biomolecules [12,13]. Hydroxybenzaldehyde derivatives serve as efficient and versatile precursors for incorporating hydroxyl functionalities into the chalcone framework. Therefore, the strategic combination of hydroxy-substituted aromatic systems with fluorinated moieties provides a rational and promising approach for the design of novel chalcone derivatives with improved pharmacological properties [14,15].

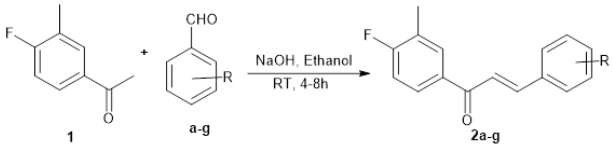
In light of the growing global concern over multidrug-resistant microbial strains, the development of new and effective antimicrobial agents remains an urgent priority [16]. Chalcone-based compounds have demonstrated considerable potential in this regard, exhibiting inhibitory activity against a broad spectrum of bacterial and fungal pathogens [17]. Accordingly, the present investigation is directed toward the synthesis and detailed characterization of a new series of chalcone derivatives derived from hydroxybenzaldehyde and fluorinated acetophenone precursors. Furthermore, their antimicrobial activities are systematically evaluated to identify promising candidates for the development of novel therapeutic agents.

II. EXPERIMENTAL METHOD:

All chemicals and solvents utilized in the present study were obtained from commercially available sources, including Sigma-Aldrich, Merck, and TCI Chemicals, and were used as received without further purification. The progress of the reactions was routinely monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} pre-coated plates (Merck), and the developed spots were visualized under ultraviolet light at wavelengths of 254 and 365 nm. Melting points were determined in open capillary tubes using a Stuart SMP30 melting point apparatus and are reported without correction. Infrared spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer equipped with an attenuated total reflectance (ATR) accessory. The 1H and 13C nuclear magnetic resonance spectra were acquired on a Bruker Avance Neo 400 MHz spectrometer using DMSO-d6 or CDCl3 as deuterated solvents, with tetramethylsilane (TMS) employed as the internal standard. Chemical shifts (δ) are reported in parts per million, and coupling constants (J) are expressed in Hertz. High-resolution mass spectrometric analyses were performed on an Agilent 6545 Q-TOF LC/MS system operating in positive electrospray ionization (ESI) mode.

III. SYNTHESIS OF CHALCONES:

The targeted fluorinated chalcone derivatives were synthesized via a Claisen–Schmidt condensation reaction employing a slightly modified literature procedure [18,19]. In a typical synthetic protocol, equimolar amounts of the appropriate hydroxybenzaldehyde derivative (1.0 mmol) and fluorinated acetophenone (1.0 mmol) were dissolved in ethanol (15 mL) and magnetically stirred in a round-bottom flask. Aqueous sodium hydroxide solution (40%, 5 mL) was then added dropwise under controlled cooling conditions maintained at 0–5 °C. Following the addition, the reaction mixture was allowed to reach room temperature and stirred continuously for 4–8 h. The progress of the reaction was monitored by thin-layer chromatography using an n-hexane/ethyl acetate (7:3, v/v) solvent system as the mobile phase. Upon completion, the reaction mixture was poured into crushed ice containing dilute hydrochloric acid (1 M), leading to the precipitation of the crude product. The resulting solid was isolated by vacuum filtration, thoroughly washed with cold distilled water, and subsequently purified by recrystallization from ethanol to yield the desired chalcone derivatives in crystalline form.



Scheme 1: Preparation of fluorinated chalcones

IV. DISC-DIFFUSION METHOD:

The in vitro antimicrobial activity of the synthesized compounds (2a–2g) was evaluated against a representative panel of microbial strains, including

Gram-positive bacteria (Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633), Gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853), and the fungal pathogen Candida albicans ATCC 10231. The assessment was performed using the broth microdilution method in accordance with the guidelines established by the Clinical and Laboratory Standards Institute [20,21].

Bacterial cultures were incubated overnight in Mueller–Hinton broth (MHB) at 37 °C, whereas the fungal strain was cultivated in Sabouraud dextrose broth (SDB) at 30 °C. The microbial inocula were standardized to a concentration of approximately 1 × 10^6 colony-forming units per milliliter (CFU/mL). The synthesized compounds, along with standard antimicrobial agents—Ciprofloxacin for bacterial strains and Fluconazole for the fungal strain—were dissolved in dimethyl sulfoxide (DMSO) and subjected to twofold serial dilutions in the համապատասխան culture media using 96-well microtiter plates. The final concentration of DMSO was maintained below 1% (v/v), a level confirmed to exert no inhibitory effect on microbial growth.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound that completely inhibited visible growth of the microorganisms after incubation at 37 °C for 24 h in the case of bacterial strains and at 30 °C for 48 h for C. albicans. All experiments were performed in triplicate to ensure the accuracy and reproducibility of the obtained results.

V. RESULTS AND DISCUSSION:

The infrared spectra of compounds displayed well-defined absorption bands characteristic of the chalcone framework. Intense bands observed in the range of 1652–1669 cm^{-1} were attributed to the stretching vibration of the α, β-unsaturated carbonyl (C=O) group, serving as a key diagnostic feature of chalcone derivatives [22,23]. Additional absorptions in the region of 1580–1595 cm^{-1} were assigned to C=C stretching vibrations, confirming the presence of a conjugated enone system.

Broad bands appearing between 3211 and 3344 cm-1 were ascribed to phenolic Ar–OH stretching vibrations. Variations in these absorptions reflected differences in the number and positional arrangement of hydroxyl groups and suggested the involvement of intra- and intermolecular hydrogen bonding interactions. Furthermore, bands observed in the range of 1225–1254 cm-1 were assigned to C–F stretching vibrations, substantiating the incorporation of fluoroaryl moieties within the molecular framework [24]. The absorptions detected between 742 and 866 cm-1 corresponded to out-of-plane aromatic C–H bending modes, consistent with di- and tri-substituted benzene ring systems.

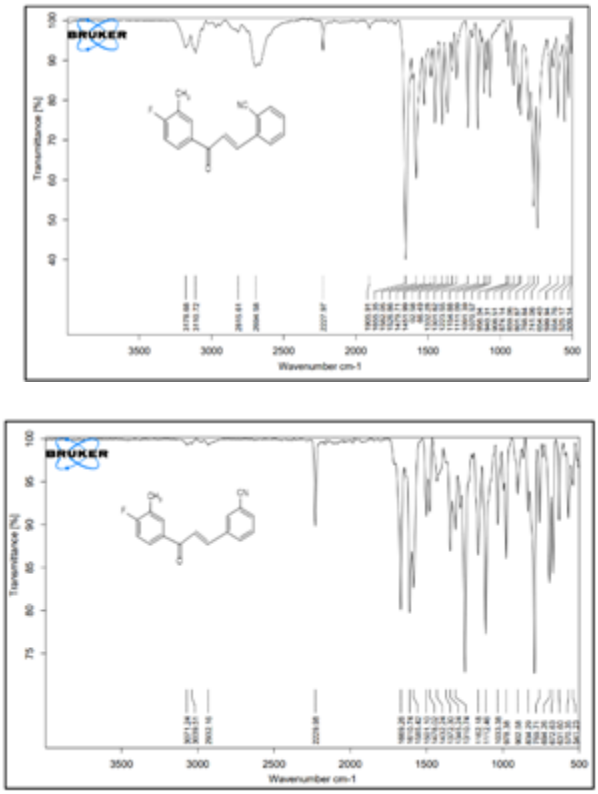


Figure 1: FTIR spectrum of compounds 2a and 2b

The 1H NMR spectra of compounds revealed characteristic resonances associated with chalcone derivatives, particularly those corresponding to the olefinic protons of the α, β-unsaturated carbonyl system. These vinylic protons consistently appeared as well-defined doublets with large coupling constants (J = 14–16 Hz), unequivocally confirming the trans (E)-configuration of the enone linkage [25]. The methyl group present on the 4-fluoro-3-

methylphenyl ring was observed as a singlet in the δ 2.32–2.37 ppm region.

Aromatic proton signals were detected as multiplets within the δ 7.1–7.9 ppm range, consistent with substituted phenyl rings. Notably, compounds bearing dihydroxy substitution exhibited significant downfield shifts, which can be attributed to deshielding effects arising from intramolecular hydrogen bonding, particularly when hydroxyl groups are positioned ortho to the enone functionality [26].

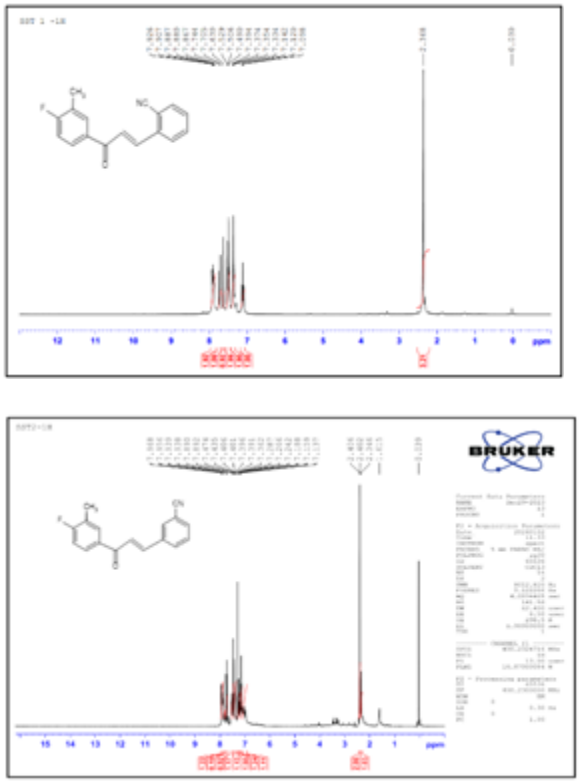


Figure 2: NMR spectrum of compounds 2a and 2b

High-resolution mass spectrometric analysis of all synthesized compounds showed molecular ion peaks in close agreement with the calculated $[M+H]^+$ values, thereby confirming their molecular compositions. For example, compound 2a exhibited a prominent ion peak at m/z 257.1457, consistent with its theoretical mass. Similarly, dihydroxy-substituted derivatives displayed characteristic molecular ion peaks around m/z 273, corresponding to the incorporation of an additional oxygen atom into the chalcone scaffold. The excellent agreement between experimental and calculated values strongly supports the proposed structures.

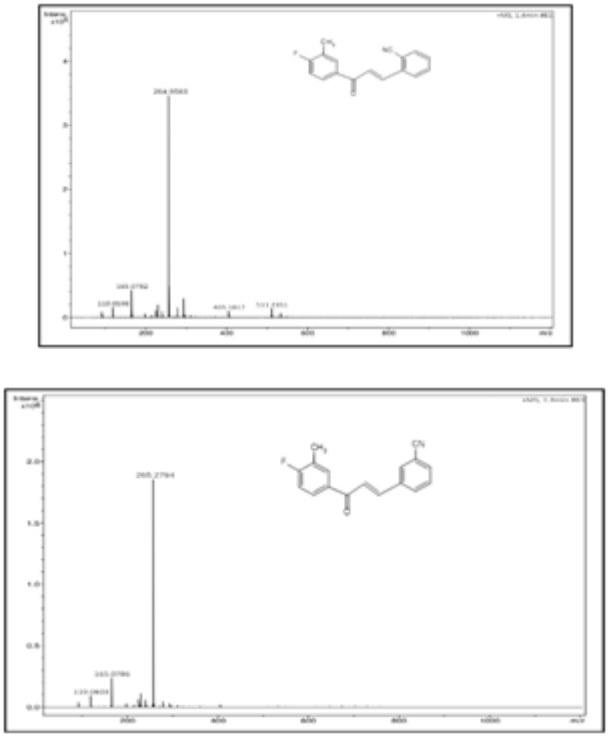


Figure 3: Mass spectrum of compounds 2a and 2b

Elemental analysis further corroborated the assigned molecular formulas, with the experimentally determined percentages of carbon, hydrogen, fluorine, and oxygen deviating by no more than ±0.4% from the theoretical values, indicating high purity of the synthesized compounds.

A comparative evaluation of hydroxyl substitution patterns revealed notable differences in the physicochemical properties of the chalcone derivatives. Monohydroxy-substituted compounds exhibited relatively lower melting points (177–181 °C), whereas dihydroxy-substituted analogues showed higher melting points in the range of 192–199 °C. This increase can be attributed to enhanced intermolecular hydrogen-bonding interactions resulting from the presence of additional hydroxyl groups [27].

In particular, ortho-dihydroxy-substituted derivatives demonstrated pronounced hydrogen-

bonding effects, as evidenced by shifts in infrared stretching frequencies and significant downfield movements in NMR signals. Moreover, the presence of a para-fluorine substituent was found to contribute to the stabilization of the conjugated enone system through increased electron delocalization, a feature commonly exploited in the rational design of biologically active chalcone derivatives [28,29].

VI. ANTIBACTERIAL ACTIVITY:

The antibacterial activity of the compounds was systematically evaluated against representative Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains using the agar well diffusion method, with streptomycin as the standard reference drug.

The results demonstrated that most synthesized chalcone derivatives exhibited moderate to significant antibacterial activity relative to the standard antibiotic. Among the Gram-positive organisms, S. aureus showed the highest susceptibility toward compound 2c, which produced a zone of inhibition of 14 mm, followed by compound 2g (11 mm), both exceeding the activity of streptomycin (10 mm). A similar trend was observed for B. subtilis, where compound 2g exhibited the maximum inhibitory effect (14 mm). These observations suggest that specific substituent patterns within the chalcone framework may enhance interactions with cellular targets such as bacterial enzymes or cell wall components [30,31].

In contrast, the synthesized compounds displayed comparatively stronger antibacterial activity against Gram-negative bacterial strains. Compounds 2c (20 mm), 2a (17 mm), and 2b (18 mm) showed pronounced inhibitory effects against E. coli, surpassing the inhibition zone of streptomycin (12 mm). Likewise, the growth of P. aeruginosa was effectively inhibited by compounds 2f (19 mm) and 2a (18 mm), while compounds 2c (14 mm) and 2g (16 mm) also exhibited appreciable antibacterial activity. The enhanced efficacy against Gram-negative bacteria may be attributed to improved membrane permeability or interactions with intracellular targets such as DNA gyrase and topoisomerase enzymes, as reported for structurally related analogues [32,33].

Interestingly, compound 2e displayed negligible activity against E. coli but showed considerable inhibition against P. aeruginosa (17 mm), indicating a degree of selectivity in its antibacterial profile. This behavior highlights the critical role of subtle structural modifications in influencing microbial susceptibility, in agreement with previous studies emphasizing the impact of electronic and positional effects of substituents on antibacterial activity [34,35].

Comparative analysis identifies compounds 2c, 2a, and 2f as the most potent antibacterial candidates, particularly against Gram-negative pathogens. The observed activities, in several cases exceeding that of Streptomycin, underscore their potential for further structural refinement and in-depth mechanistic exploration.

VII. ANTIFUNGAL ACTIVITY:

The antifungal potential of the synthesized chalcone derivatives was investigated against Candida albicans and Saccharomyces cerevisiae using the agar well diffusion method, with Fluconazole employed as the standard reference agent.

Among the evaluated compounds, derivative 2e exhibited the highest antifungal activity, producing inhibition zones of 17 mm against C. albicans and 18 mm against S. cerevisiae, thereby surpassing the activity of Fluconazole, which showed corresponding inhibition zones of 13 and 12 mm. Additionally, compounds 2b and 2g demonstrated considerable antifungal efficacy, with inhibition zones ranging from 16 to 17 mm against both fungal strains. These findings indicate that specific substituent arrangements within the chalcone framework may promote enhanced interactions with fungal cell membranes or essential enzymatic systems, resulting in improved antifungal performance.

In contrast, compounds 2f and 2c exhibited moderate antifungal activity, with inhibition zones in

the range of 12–14 mm. Although their activity against C. albicans was comparable to or slightly higher than that of the reference drug, reduced efficacy was observed against S. cerevisiae. This comparatively lower activity may be attributed to less favorable electronic or steric characteristics of the substituents, which could influence membrane permeability and target binding affinity.

The antifungal evaluation highlights the significant role of structural modifications in determining activity profiles within this series. Notably, compounds 2b, 2d, 2e, and 2g emerged as promising candidates, exhibiting potent and broad-spectrum antifungal activity. These observations are consistent with earlier reports indicating that substituted aromatic and heteroaryl systems can enhance antifungal efficacy through mechanisms such as disruption of ergosterol biosynthesis or inhibition of fungal cytochrome P450 enzymes [36,37].

In conclusion, the synthesized chalcone derivatives—particularly compounds 2e and 2b—represent promising antifungal leads and merit further structural optimization and detailed mechanistic exploration.

VIII. CONCLUSION:

A systematic synthetic methodology was successfully implemented for the preparation of a series of fluorine- and hydroxyl-substituted chalcone derivatives, whose structures were unequivocally confirmed through detailed spectroscopic and analytical investigations. All synthesized compounds were obtained as white crystalline solids in good to excellent yields (77–87%) and exhibited sharp melting points, indicative of a high degree of purity. The FT-IR spectra displayed characteristic absorption bands corresponding to phenolic hydroxyl (Ar–OH), α, β-unsaturated carbonyl (C=O), olefinic (C=C, –CH=), and C–F stretching vibrations, thereby verifying the presence of the desired functional groups within the molecular framework.

The 1H NMR spectra provided further confirmation of the proposed structures, revealing distinctive downfield signals associated with the α,β-unsaturated enone protons, along with resonances attributable to aromatic protons and methyl substituents, consistent with the chalcone scaffold. High-resolution mass spectrometric analysis exhibited molecular ion peaks in close agreement with the calculated m/z values, while elemental analysis results were within acceptable deviations from theoretical values, collectively supporting the proposed molecular compositions.

The combined spectroscopic, mass spectrometric, and elemental analyses conclusively validate the successful synthesis of the target chalcone derivatives. The deliberate incorporation of hydroxyl groups at varying positions on the aromatic rings, together with fluorine substitution, introduces significant structural diversity, which is expected to influence their physicochemical properties and biological profiles. Consequently, these compounds represent promising candidates for further pharmacological investigation.

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