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Synthesis, and *in-vitro* biological evaluation of chalcone derivatives as antimicrobial agents

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Abstract: The rising resistance to antimicrobial drugs has highlighted the urgent need for discovering novel compounds with diverse mechanisms of action that can target both sensitive and resistant strains. To address this, we developed some chalcone analogs. In this study, a series of compounds (12-15) featuring fluoro and trifluoromethyl groups on the B ring were synthesized and evaluated for their antimicrobial properties. The positions of the substituents on the B ring were altered to assess their impact on antimicrobial activity. Compounds 13 and 14 demonstrated potent antibacterial activity (MIC = 15.6 and 7.81 µg/mL, respectively) against *Staphylococcus aureus*. Overall, our findings highlight Compounds 13 and 14 as promising scaffolds warranting further optimization for the development of effective antibacterial agents.

Keywords: Chalcone; synthesis; antimicrobial activity; MIC; SAR.

1. Introduction

Bacterial infections present a significant challenge due to the increasing multi-drug resistance exhibited by microbial pathogens, a phenomenon not limited to bacteria but also affecting other pathogens such as parasites, fungi, and viruses. This rapid emergence of antimicrobial resistance has driven researchers to explore new compounds with effective antimicrobial properties.[1-4]

Chalcones serve as precursors to flavonoids, characterized as linear flavonoids with three carbon chains (an α,β-unsaturated carbonyl system) linking two aromatic rings.[5,6] Their significance lies in their diverse biological activities, which include antimicrobial, cytotoxic, antidiabetic, anti-inflammatory, antioxidant, antiulcer, and antitubercular properties.[7-9] Chalcones are compounds with simple chemistry that offer easy synthetic access to various substituted derivatives. Chalcones can be easily synthesized via the Claisen–Schmidt reaction of acetophenones and benzaldehydes under basic conditions.[10]

In recent years, compounds containing fluorine have become common as potential lead drugs.[11] It has been reported that insertion of a fluorine atom into a biologically active compound results in minimal steric change, thus maintaining interactions with enzyme active sites, receptor recognitions sites, and other biological systems.[12] Furthermore, it has been indicated that the high electronegativity of fluorine can lead to significant changes in the physical and chemical properties of the molecule.[13] It has been established in recent literature that fluorine-substituted compounds exhibit improved bioactivity and efficacy. Compounds with a chalcone backbone have also been reported to exhibit antimicrobial activity.[6,14–16]

A series of chloro-fluorine-containing hydroxy pyrazolines, whose antibacterial activities were evaluated using the disk diffusion method, as reported by Karthikeyan and colleagues. Their findings

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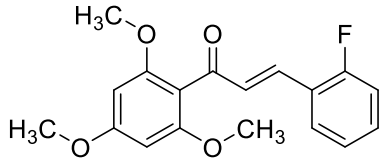
indicated that, due to the favorable effects of halogens, the tested compounds exhibited strong bacterial inhibition.[17]

In another study, Batovska and collaborators emphasized the importance of the lipophilicity of the A ring, noting that the presence of a para-electron-withdrawing group in the B ring enhances activity, especially when the A ring contains a methoxy group.[18] Burmaoglu and his team synthesized various chalcones featuring saturated or semi-saturated linkers and fluorine substitutions on the B ring, identifying compound A as the most potent derivative against *S. aureus* among those tested.[2]



Compound A

In this study, we performed antimicrobial activity studies on six B-ring fluoro or trifluoromethyl-substituted chalcones and one nonsubstituted chalcones that we have previously synthesized in an efficient, high-yielding manner[2,13]. These activity studies indicated that they may be used in the treatment of bacterial infections.

2. Experimental

2.1. Chemical Material and Apparatus

Commercial grade reagents and solvents were used without further purification. The purity of all compounds was judged by TLC analysis (single-spot/two-solvent systems) using a UV lamp. Melting points were measured with Gallenkamp melting point devices. ^{1}H NMR and ^{13}C NMR spectra were taken on 400 and 100 MHz spectrometer with tetramethyl silane (TMS) as an internal standard, and chemical shifts were recorded in ppm values. The IR spectra were obtained on Perkin Elmer Spectrum One FT-IR spectrometer.

2.2. Biological Materials and Apparatus

The compounds 9-15 were tested against *Staphylococcus aureus* (ATCC 29213), *S. pneumoniae* (ATTC 49619), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231), and *Candida parapsilosis* (ATTC 22109).

2.3. Chemistry

Non-fluoro, fluoro and trifluoromethyl-substituted chalcones compounds were synthesized and showed different activities as described previously (Scheme 1).[2, 11, 13]

*2.3.1. Synthesis of Chalcone Compounds (**9-12**)*

To a solution of 2,4,6trimethoxyacetophenone (1 eq.) (1) in MeOH (2.5 mL/1 mmol of substrate) benzaldehyde derivatives (2-5) (1,6 eq.) and % 50 KOH solution (1.5 mL/1 mmol of substrate) was added sequentially and stirred for 15 h at room temperature. After 15 h solvent was evaporated. Crude material exracted with 2M HCl solution (2 mL/1 mmol of substrate) and DCM (2 mL/1 mmol of substrate x 3). The combined extracts were dried over Na_2SO_4. The solvent was removed in vacuo and the remaining residue purified via coloumn chromatography over silica gel using gradient elution with EtOAc and hexanes to yield compounds **9**-**12**.

*(E)-3-Phenyl-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**9**)*:[13] The above procedure was followed with benzaldehyde (2) to yield 9 as a yellow solid (70% yield). Rf (EtOAc/Hexanes 30:70): 0.3, m.p: 90°C.

*(E)-3-(2-Fluorophenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**10**):*[13] The above procedure was followed with 2-fluorobenzaldehyde (3) to yield 10 as a yellow solid (72% yield). Rf (EtOAc/Hexanes 30:70): 0.3, m.p:105-107°C.

*(E)-3-(3-Fluorophenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**11**):*[13] The above procedure was followed with 3-fluorobenzaldehyde (4) to yield 11 as a yellow solid (81% yield). Rf (EtOAc/Hexanes 30:70):0.3, m.p: 89-91°C.

*(E)-3-(4-Fluorophenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**12**):*[2] The above procedure was followed with 4-fluorobenzaldehyde (5) to yield 12 as a yellow solid (70% yield). Rf (EtOAc/Hexanes 30:70):0.3, m.p: 120-122°C.

*2.3.2. Synthesis of chalcone compounds (**13-15**)*

To a solution of 2,4,6-trimethoxyacetophenon (1) (1.5 equiv.) in THF: H_2O (5 : 1, 10 mL), flouro and triflouromethyl substituted benzaldehyde derivatives (6–8) (1 equiv.) and $LiOH.H_2O$ (10 equiv.) were added sequentially and stirred stirred overnight at room temperature. The reaction was terminated by TLC after control, the mixture was quenched with saturated NH_4Cl solution (5 mL) and extracted with ethyl acetate (EtOAc) (25 mL×3). The combined organic layers were dried over Na_2SO_4. The solvent was removed in a vacuum and the remaining residue was purified via column chromatography over silica gel using gradient elution with EtOAc and hexanes to yield compound 13-15.

*(E)-3-(2-(Trifluoromethyl)phenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**12**):*[11] The above procedure was followed with compound 2-(trifluoromethyl)benzaldehyde (6) to yield 12 as a yellow solid (65% yield). Rf (EtOAc/Hexanes 30: 70): 0.4, m.p: 143-145°C.

*(E)-3-(3-(Trifluoromethyl)phenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**13**):*[11] The above procedure was followed with compound 3-(trifluoromethyl)benzaldehyde (7) to yield 13 as a light yellow solid (69% yield). Rf (EtOAc/Hexanes 30: 70): 0.4, m.p: 121-123 °C.

*(E)-3-(4-(Trifluoromethyl)phenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**14**):*[11] The above procedure was followed with compound 4-(trifluoromethyl)benzaldehyde (8) to yield 14 as a light yellow solid (80% yield). Rf (EtOAc/Hexanes 30: 70): 0.5, m.p: 101-102 °C.

2.4. Antimicrobial Activity Studies

Antimicrobial activities of the compounds were performed by modifying methods in the literature.[19, 20]

The fungal and bacterial cell inoculum were prepared from a stock culture grown in tryptic soy agar (TSA) at 28 ˚C for 24 h, and Mueller–Hinton agar (MHA) at 37 ˚C for 24 h, respectively. The microorganism suspension concentrations were adjusted according to McFarland 0.5 turbidity tubes using sterilized saline. Stock solutions of the synthesized compounds (**9**-**15**) were prepared in DMSO at 1000 mg/mL. A modified microdilution test was applied for antimicrobial activity, and the experiments were run in duplicate independently.

For antifungal activity testing, 100 mL Tryptic Soy Broth (TSB) was added to each of the 11 wells. A 100 mL aliquot of the tested chemical solution was added to the first well, and twofold dilutions were prepared. Then, 5 mL of fungal suspension was added to each well except the last one, which acted as the control well.

For antibacterial activity testing, 100 mL Mueller–Hinton broth (MHB) was added to each of the 11 wells. A 100 mL aliquot of the chemical derivative solution was added to the first well, and twofold dilutions were prepared. Then, 5 mL of the bacterial suspension was added to each wells, except the last control well. A control well containing 5 mL of the fungal and bacterial suspensions alone without the tested compounds was also prepared. All plates were incubated at 28 ˚C (for fungi) and at 37 ˚C (for bacteria) for 24 h. After incubation, the MICs (Table 1) were obtained by noting the growth inhibitions.

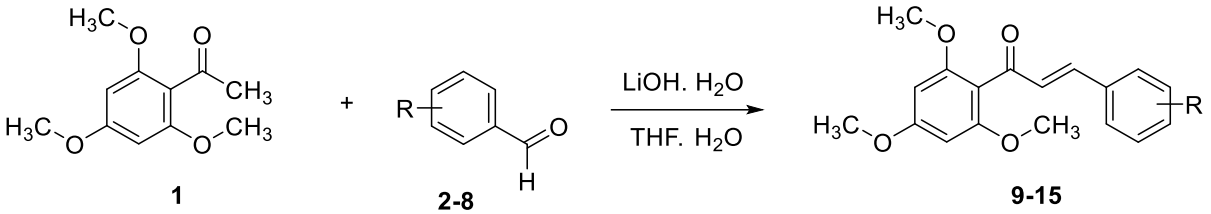
The concentration resulting in a 50% reduction in the optical density (OD) values was compared to a reproduction control at 450nm by spectrophotometric evaluation and defined as the MIC value. Fluconazole and ampicillin were used as reference drugs. The results were read visually and by measuring optical density for 24 h.

3. Results and Discussion

3.1. Chemistry

All compounds, B-ring fluoro or trifluloro-substituted chalcone derivatives, were previously synthesized by other groups.[2,11,13] We report here the synthesis of compounds and their structural details. The general synthesis method and structure of synthesized compounds are shown in Scheme 1.

Compounds **9**-**15** were synthesized in a single step using the Claisen-Schmidt condensation reaction. This was accomplished by reacting 2,4,6 trimethoxy acetophenone (**1**) with fluoro and trifluoromethyl substituted benzaldehyde derivatives (**2**–**8**) in the presence of LiOH, a preferred aqueous alkali base. The reaction solvent was identified as a tetrahydrofuran: water (THF: H_2O) mixture.[10] Trimethoxy chalcone derivatives (**9**-**15**) were isolated in high yield. The synthesized compounds were fully characterized with common spectroscopy techniques using ^{1}H-NMR, ^{13}C-NMR, and IR (Scheme 1).



2; R = H, **3**; R = 2-F, **4**; R = 3-F, **5**; R = 4-F,
6; R = 2-CF_3, **7**; R = 3-CF_3, **8**; R = 4-CF_3

Scheme 1. General structures of synthesized compounds

Modification of compound **9** (the methoxy derivative with non substituent) for activity modulation was made through preparation of compounds with fluoro (**10**-**12**) or trifluoromethyl substituents (**13**-**15**) on the B ring. The synthesized compounds were fully characterized with common spectroscopy techniques. To evaluate the potential of the basic pharmacophore of chalcone, **9** was evaluated for its antimicrobial efficacy against bacteria and fungi. The different functional groups carrying on aromatic rings of chalcones are responsible for their antimicrobial activities. The details of the substitution patterns in the target compounds are presented in Table 1.

3.2. Antimicrobial Activity Studies

In this study, the antimicrobial activity of seven synthesized compounds was tested using the microdilution method with reference to the MIC values of the compounds against four reference bacterial and two reference yeast strains.

The antibacterial activities of the target compounds (**9**–**15**) against the four common pathogenic bacterial strains *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 is shown in Table 1. The well-known commercial antibiotic ampicillin was used as the standard drug during the assay.

The results showed that the compounds were able to inhibit the growth of tested microorganisms in the MIC range of 7.81–250 µg/mL. In particular, compounds **9**, **13**, **14** and **15** were as effective as the reference drug (ampicillin) against tested bacteria strains (Table 1).

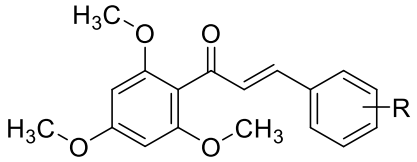
Compounds **13** and **14** showed significant activity against almost all the bacterial strains, and compound **14** is the most active compound against *S. aureus*. Interestingly, all the compounds derived from trifluoromethyl-substituted chalcone scaffolds exhibit significant inhibition activities against all the tested strains, while the other compounds exhibit moderate and low activity.

Compounds were evaluated for antifungal activity against the *C. albicans*, and *C. parapsilosis* fungal strains. The MIC values obtained for the compounds are given in Table 1. Fluconazole was used as the reference for inhibitory activity against fungi. The antifungal activities of all the compounds are comparable to that of fluconazole.

Compounds **9**, **13-15** are the most active compounds, with compound **13** exhibiting excellent activity against fungi strains. Compounds **9**, **13**, and **14** exhibit very good activity against *C. albicans*- at an MIC of 15.6 to 31.25 mg/mL, while the other compounds (**10-12**, and **15**) exhibit moderated inhibition with MICs of 62.5 and 250 mg/mL, as compared to the standard fluconazole.

Compounds **13-15** exhibit the highest activity against *C. parapsilosis*, with MICs of 15.6 to 31.25 mg/mL, whereas all other compounds exhibit low inhibitory activity compared to that of fluconazole, with MICs of 62.5-250 mg/mL. Compound **13**, the trifluoromethyl-substituted chalcone analog, exhibits the highest activity.

Table 1. MIC's of compounds **(9-15**) and the standart compounds against the selected bacteria and fungi strains



Compound No	R_1	*S. aureus*	*S. pneumoniae*	*E. coli*	*P. aeruginosa*	C. albicans	*C. parapsilosis*	*cLogP*[a]
9	H	31.25	125	31.25	62.5	15.6	62.5	3.82
10	2-F	62.5	62.5	62.5	62.5	250	125	3.96
11	3-F	125	62.5	125	125	125	62.5	3.96
12	4-F	62.5	125	125	125	62.5	62.5	3.96
13	2-CF_3	**15.6**	62.5	31.25	31.25	**15.6**	**15.6**	4.70
14	3-CF_3	**7.81**	31.25	62.5	31.25	31.25	31.25	4.70
15	4-CF_3	31.25	62.5	31.25	62.5	62.5	31.25	4.70
Fluconazole		-	-	-	-	*	*	
Ampicillin		*	*	31.25	31.25	-	-	

[a] *cLogP* value of the synthesized compounds calculated using ChemBioDraw Ultra 12.0.3

* All tested concentrations are active - Not tested. Results in bold indicate the compounds with the highest selective index

3.3. SAR Studies

The SAR of compounds **9-15** was explored using the data presented in Table 1, and reveals that the presence of three methoxy groups on the A ring and fluoro atoms of the chalcone increases antimicrobial activity. Chalcone compounds exhibit more pronounced antimicrobial activity.

More specifically, compounds with fluoro or trifluoromethyl substituents in position 2 of the B ring in the chalcone structure exhibit significantly increased potency against the microbial strains. The influence of Log p on the antifungal activity of the compounds appears to be important: as Log p increases, the antifungal activity increases.

4. Conclusion

The emergence of multidrug-resistant bacterial pathogens underscores the urgent need for novel antimicrobial agents. Our study focused on the evaluation of six B-ring fluoro or trifluoromethyl-substituted chalcones and one nonsubstituted chalcone, synthesized efficiently with high yields, for their potential antibacterial properties. These findings support the hypothesis that fluorine substitutions on the chalcone backbone enhance antibacterial activity by improving bioactivity and efficacy, consistent with previous reports on the beneficial role of halogens in antimicrobial compounds.

In this study, based on the recent literature data, we synthesized and tested a series of 2,4,6-trimethoxy and non/mono or trifluoromethyl-substituted chalcone derivatives in order to improve the antimicrobial activity of previously synthesized chalcones and to gain an understanding of their SAR in the context of antimicrobial activities (Table 1).

The synthetic route for chalcone derivatives (**9**–**15**) is represented in Scheme 1. The seven chalcone derivatives were prepared via one step. The compounds tested for antimicrobial activity were divided into two series; series A comprised trimethoxy- substituted compounds **9**-**12** derived from the parent structure 3 with fluoro substituents in position 2 of the B ring, and series B contained three trifluormethyl substituted on the B ring (**13**–**15**).

Modification of compounds for activity modulation was made by introducing trifluomethyl group. These compounds were found to be more active. It is widely known that fluoro group plays an important role in drug–protein interactions. However, we found that the conformationally restricted chalcones were more active than the other chalcones.

This modification was made in such a way that the total lipophilic nature of the compounds was similar (The Log p values for compounds are shown in Table 1). We also attempted to assess the importance of the structure of the chalcone compounds to their effectiveness as antimicrobial agents.
The synthesized compounds were also evaluated for their *in-vitro* antimicrobial activity against several bacterial and fungal strains. The tested compounds were active against bacteria and fungi, and they did exhibit significant antibacterial and antifungal activities, especialy against *S. aureus*, in comparison to ampicillin and fluconazole. Interestingly, compounds **13** and **14** exhibited wider activity than the other compounds.

In conclusion, the evaluation of the biological activity of these molecules showed that chalcones with a conformationally higher number of fluorine atoms, analogues, are more active and may be good for the future development of antimicrobial drugs.

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