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Synthesis and Antimicrobial Evaluation of a Series of Chlorinated Chalcone Derivatives

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ABSTRACT

A systematic study of chlorinated chalcones was used to determine the antimicrobial effects of varying the location of the halogen substituent on each aromatic ring of the chalcone. Sixteen chalcones were synthesized by Claisen-Schmidt condensation of substituted acetophenones and benzaldehydes. In some instances, employing a solvent-free procedure produced higher yields with enhanced purity. Antimicrobial activity of the synthesized chalcones was assessed using agar well diffusion assay by measuring the zones of inhibition against pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). The results show that nearly all the chlorinated chalcones synthesized display antibacterial activity comparable to sulfanilamide and showcases the potential for substituted chalcones to serve as new antimicrobial agents.

INTRODUCTION

Treatment of bacterial infection is a major emerging health issue. New infectious diseases are discovered every year, including an increase in the numbers of antibiotic-resistant pathogens. To address this dilemma, it is critical to develop new compounds with antibacterial properties. Chalcones, of the flavonoid family, are characterized by their dual aromatic rings and α,β-unsaturated ketone moiety which confers their potent antimicrobial activity [1-8]. In addition to antimicrobial activity, chalcones have been shown to exhibit a multitude of other pharmacological activities including, but not limited to, antioxidant [9,10], anti-inflammatory [11-13], and antitumor [14-17]. A systematic synthesis and biological evaluation of the chlorine positioning was needed to probe deeper into structure-activity relationships.

This study presents a full series of 16 chalcones with varied placement of chlorine atoms on the ortho, meta, and para positions of both aromatic rings. To achieve this, a Claisen-Schmidt condensation of a benzaldehyde (**1a-d**) and an acetophenone (**2a-d**) was employed with sodium hydroxide in ethanol. All synthesized compounds were characterized by melting point, thin layer chromatography (TLC), and infrared spectrum analysis (IR). The antibacterial activity of each chalcone (**3a-p**) was assessed using agar well diffusion assay by measuring the zone of inhibition with 100 µg of sample. The goals of this study were to synthesize a series of chlorinated-chalcones and to probe the effect of the chlorine substituent location on antimicrobial activity.

MATERIALS AND METHODS

Chemicals and Equipment

All the starting materials including acetophenones, benzaldehydes, sodium hydroxide and solvents used in this study were analytical grade and used in reactions as received without further purification (Fisher Scientific). IR spectra were recorded on a ThermoFisher FT-IR. Melting points were determined in open capillaries using a Mel-Temp machine.

General Procedure for Synthesis of Chalcones 3a, 3c-3e, 3g-3i, 3k-3m, 3o-3p

NaOH (3.75 mmol, 15 M) was added to a round bottom flask containing benzaldehyde (2.50 mmol), acetophenone (2.50 mmol), and ethanol (2 mL, 200 proof). The mixture was stirred at room temperature for 30-60 minutes (monitored by TLC in 10-20% EtOAc/Hexanes) during

which a precipitate formed. The mixture was cooled to 0°C and collected via vacuum filtration, washing with ice-cold ethanol, and allowed to dry under vacuum for 15 minutes. The crude product was purified by recrystallization from hot ethanol and air-dried overnight to yield pure chalcone.

Solvent-free Procedure for Synthesis of Chalcones 3b, 3f, 3j, 3n

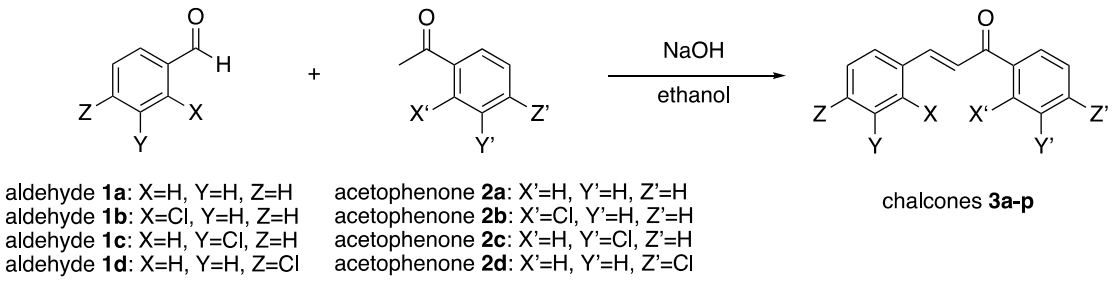
Powdered NaOH (3.75 mmol) was added to a mortar containing benzaldehyde (2.50 mmol) and acetophenone (2.50 mmol). The mixture was ground with a pestle at room temperature until complete (monitored by TLC in 10-20% EtOAc/Hexanes). The resulting paste was washed with ice-cold water, followed by ice-cold ethanol, and then collected via vacuum filtration. The crude product was purified by recrystallization from hot ethanol and air-dried overnight to yield pure chalcone.

Antibacterial Activity Evaluation

The antimicrobial activity of the 16 synthesized chalcones was tested against the bacterial species *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* using an agar well diffusion assay [18,19]. Broth cultures were prepared for each of the three bacterial species and standardized to an optical density of 1.0 measured at 600 nm. A bacterial lawn was prepared on Mueller-Hinton agar plates for subsequent well diffusion analysis. Wells of 9 mm diameter were punched in each plate using a sterile borer. 100 µL of each test compound (1 mg/mL in DMSO) was pipetted into their corresponding wells and incubated at 37°C overnight. Sulfanilamide was used as a positive control, and DMSO was used as a negative control. Zones of inhibition were measured to determine antimicrobial activity of the synthesized compounds, and their effectiveness with respect to each bacterial species.

RESULTS AND DISCUSSION

The synthesis of chalcones **3a-p** was achieved via Claisen-Schmidt condensation of aromatic aldehydes **1a-d** with acetophenones **2a-d** (Scheme 1).



Scheme 1: Synthesis of chalcones

It was discovered that certain chalcones were formed in lower yields with standard conditions, so a solvent-free procedure [20] was employed, resulting in higher yields and purity. Yields for all sixteen chalcones are shown in Figure 1.

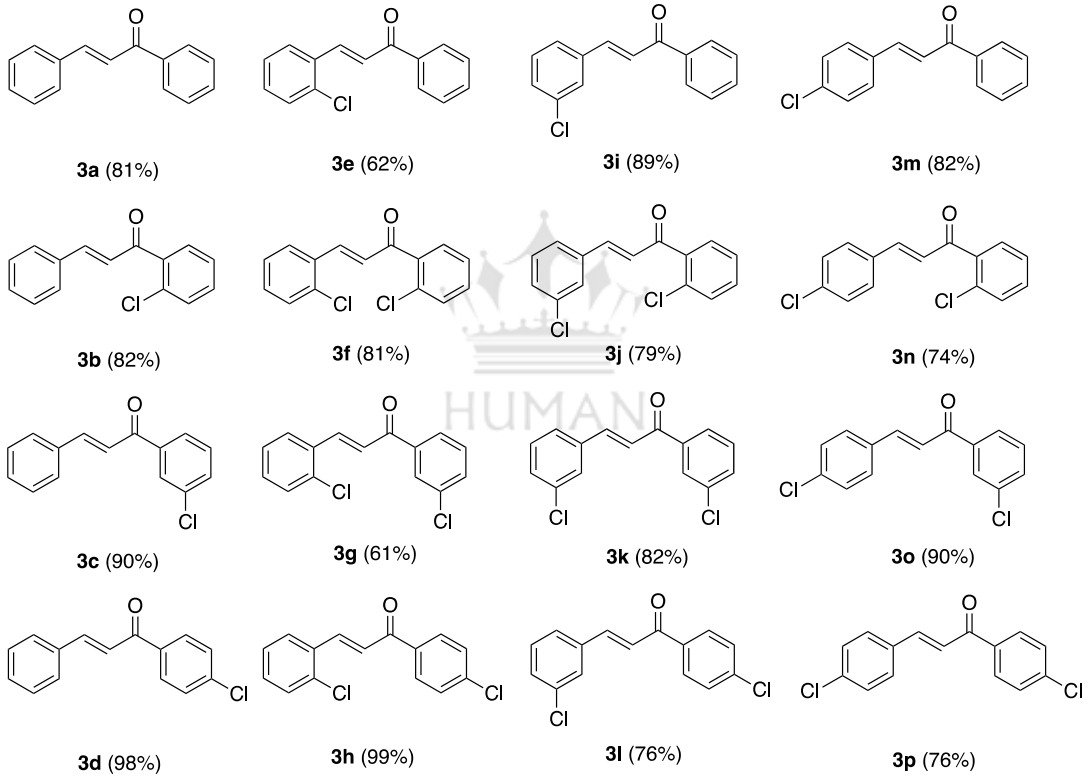


Figure 1: Synthesized chalcones 3a-p

1,3-diphenyl-2-propen-1-one (**3a**): mol. formula: $C_{15}H_{12}O$; yield = 0.422 g, 2.03 mmol, 81%; mp = 46.6-47.4°C; FT-IR (KBr cm^{-1}): 1659 (C=O), 1601 (C=C).

1-(-2-chlorophenyl)-3-phenyl-2-propen-1-one (**3b**): mol. formula: $C_{15}H_{11}OCl$; yield = 0.496 g, 2.04 mmol, 82%; mp = oil; FT-IR (KBr cm^{-1}): 1647 (C=O), 1597 (C=C), 827 (Ar-Cl).

1-(-3-chlorophenyl)-3-phenyl-2-propen-1-one (**3c**): mol. formula: $C_{15}H_{11}OCl$; yield = 0.545 g, 2.25 mmol, 90%; mp = 92.0-93.8°C; FT-IR (KBr cm^{-1}): 1661 (C=O), 1600 (C=C), 865 (Ar-Cl).

1-(-4-chlorophenyl)-3-phenyl-2-propen-1-one (**3d**): mol. formula: $C_{15}H_{11}OCl$; yield = 0.596 g, 2.46 mmol, 98%; mp = 96.6-97.0°C; FT-IR (KBr cm^{-1}): 1660 (C=O), 1595 (C=C), 827 (Ar-Cl).

3-(2-chlorophenyl)-1-phenyl-2-propen-1-one (**3e**): mol. formula: $C_{15}H_{11}OCl$; Yield = 0.378 g, 1.55 mmol, 62%; MP = 178.4-180.8°C; FT-IR (KBr cm^{-1}): 1665 (C=O), 1594 (C=C), 850 (Ar-Cl).

1-(-2-chlorophenyl)-3-(2-chlorophenyl)-2-propen-1-one (**3f**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.560 g, 2.02 mmol, 81%; mp = >250°C; FT-IR (KBr cm^{-1}): 1661 (C=O), 1595 (C=C), 828 (Ar-Cl).

1-(-3-chlorophenyl)-3-(2-chlorophenyl)-2-propen-1-one (**3g**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 421 g, 1.52 mmol, 61%; mp = 63.5-65.6°C; FT-IR (KBr cm^{-1}): 1660 (C=O), 1590 (C=C), 825 (Ar-Cl).

1-(-4-chlorophenyl)-3-(2-chlorophenyl)-2-propen-1-one (**3h**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.689 g, 2.49 mmol, 99%; mp = >250°C; FT-IR (KBr cm^{-1}): 1671 (C=O), 1588 (C=C), 840 (Ar-Cl).

3-(3-chlorophenyl)-1-phenyl-2-propen-1-one (**3i**): mol. formula: $C_{15}H_{11}OCl$; Yield = 0.539 g, 2.22 mmol, 89%; MP = 71.8-72.5°C; FT-IR (KBr cm^{-1}): 1659 (C=O), 1598 (C=C), 827 (Ar-Cl).

1-(-2-chlorophenyl)-3-(3-chlorophenyl)-2-propen-1-one (**3j**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.546 g, 1.97 mmol, 79%; mp = 55.6-59.6°C; FT-IR (KBr cm^{-1}): 1666 (C=O), 1606 (C=C), 837 (Ar-Cl).

1-(-3-chlorophenyl)-3-(3-chlorophenyl)-2-propen-1-one (**3k**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.565 g, 2.04 mmol, 82%; mp = oil; FT-IR (KBr cm^{-1}): 1669 (C=O), 1592 (C=C), 783 (Ar-Cl).

1-(-4-chlorophenyl)-3-(3-chlorophenyl)-2-propen-1-one (**3l**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.661 g, 1.89 mmol, 76%; mp = 79.8-82.0°C; FT-IR (KBr cm^{-1}): 1659 (C=O), 1600 (C=C), 824 (Ar-Cl).

3-(4-chlorophenyl)-1-phenyl-2-propen-1-one (**3m**): mol. formula: $C_{15}H_{11}OCl$; Yield = 0.990g, 4.08 mmol, 82%; MP = 112.9-113.6°C; FT-IR (KBr cm^{-1}): 1657 (C=O), 1591 (C=C), 820 (Ar-Cl).

1-(-2-chlorophenyl)-3-(4-chlorophenyl)-2-propen-1-one (**3n**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.517 g, 1.86 mmol, 74%; mp = 183.9-186.5°C; FT-IR (KBr cm^{-1}): 1657 (C=O), 1578 (C=C), 860 (Ar-Cl).

1-(-3-chlorophenyl)-3-(4-chlorophenyl)-2-propen-1-one (**3o**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.626 g, 2.26 mmol, 90%; mp = 131.6-133.5°C; FT-IR (KBr cm^{-1}): 1659 (C=O), 1587 (C=C), 821 (Ar-Cl).

1-(-4-chlorophenyl)-3-(4-chlorophenyl)-2-propen-1-one (**3p**): mol. formula: $C_{15}H_{10}OCl_2$; yield = 0.524 g, 1.89 mmol, 76%; mp = 155.8-156.6°C; FT-IR (KBr cm^{-1}): 1654 (C=O), 1584 (C=C), 834 (Ar-Cl).

General trends in the data show reduced yields for chalcones derived from using 2-chlorobenaldehyde, which may be attributed to the steric hinderance of the *ortho*-substituent. In contrast, yields for the para-substituted benzaldehyde were generally the highest, presumably due to the electron withdrawing ability of the electronegative chlorine atom.

Each synthesized chalcone was evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria (Table 1). Trends in the biological data indicate that many of the synthesized chalcones were as effective as the positive control sulfanilamide. Placement of the chlorine was shown to directly impact the antibacterial activity. This is rationalized by electronic and steric effects of the nucleophilic attack on the beta-carbon of the α,β-unsaturated ketone moiety. Differences in measured inhibition zones may also be attributed to the different bacterial strains. For *P. aeruginosa*, the 4-position and 4'-positions resulted in the most active compounds. For *S. aureus*, the 4-position and 2'-position resulted in the most active compounds. Moreover, for *E. coli*, the 2-position and 3'-position resulted in the most active compounds.

Table 1: Antimicrobial activity of the synthesized chalcones

Compound	Zone of inhibition (mm)		
	P. aeruginosa	*S. aureus*	*E. coli*
chalcone (**3a**)	13	12	12
2'-chlorochalcone (**3b**)	No activity	No activity	No activity
3'-chlorochalcone (**3c**)	13	11	11
4'-chlorochalcone (**3d**)	13	11	12
2-chlorochalcone (**3e**)	11	14	13
2,2'-dichlorochalcone (**3f**)	12	13	12
2,3'-dichlorochalcone (**3g**)	12	12	14
2,4'-dichlorochalcone (**3h**)	13	12	13
3-chlorochalcone (**3i**)	12	13	11
3,2'-dichlorochalcone (**3j**)	12	12	12
3,3'-dichlorochalcone (**3k**)	13	10	12
3,4'-dichlorochalcone (**3l**)	13	11	11
4-chlorochalcone (**3m**)	12	13	12
4,2'-dichlorochalcone (**3n**)	14	15	12
4,3'-dichlorochalcone (**3o**)	14	13	14
4,4'-dichlorochalcone (**3p**)	14	10	11
sulfanilamide	15	15	16

CONCLUSION

This study demonstrates that varying the chlorine substituent location on each aromatic ring of the chalcone backbone can affect the antimicrobial activity. All but one synthesized chalcone displayed activity against Gram-positive and Gram-negative bacteria. Further investigation will use the structure-activity trends to determine new targets of opportunity for antimicrobial agents.

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